The Journal of Laboratory and Clinical Medicine

VOL. 22 MAY, 1937 No. 8

CLINICAL AND EXPERIMENTAL

FURTHER STUDIES ON THE MECHANISM OF DIURESIS WITH ESPECIAL REFERENCE TO THE ACTION OF SOME NEWER DIURETICS*

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THERE are still sharp differences of opinion as to where and how diuretic drugs act. American investigators for the most part are agreed that the kidney is the site of the chief and preponderant pharmacologic effect. The continentals, particularly German investigators, insist upon the presence of extrarenal factors in the production of diuresis. Some postulate a "physicotherapeutic water freeing" effect upon the tissue colloids with a resulting temporary hydremia. This increase in blood volume by dilating the renal vascular bed is considered by Meyer[1] to account for the actual outpouring of urine. Jackson[2] demonstrated some pharmacodynamic effect of mersalyl on most, if not all, of the vascular bed of the dog. He pointed to a possible analogy to A. N. Richards'[3] findings of a quantitatively different action of substances, which had been found to cause less contraction of the afferent than of the efferent arterioles to and from the glomerular tufts, this leading to increased urine formation in Bowman's capsules. These theories do not admit of clinical investigative approach.

THE BLOOD SERUM PROTEIN STUDIES

Shally,[4] after some confirmatory physiochemical studies, accepted the conclusion of Nonnebruch and others that changes in the colloid osmotic pressure

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Received for publication, June 1, 1936.

Presented before the Section on Pharmacology and Therapeutics, A.M.A. May 12, 1936, at Kansas City.

did not explain the movements of water in diuresis. However, he found an absolute and relative increase in the albumin fraction with lowered and unchanged globulin and a drop in fibrinogen at the onset of diuresis in all cases. He tried the effect of mersalyl on blood serum in vitro and concluded that in the concentration that it appeared in the blood and after injection it produces no change in the serum protein, even though at higher concentration there was flocculation. However, in this country, Bieter and Wright[5] have demonstrated with their ultramicroscope changes in the blood serum of those who had received therapeutic doses of the mersalyl. Such ultramicroscopic alterations, we believe, could change the "salting out" levels of the blood protein constituents and account for Shally's shifts in the ratios. The changes in blood protein values that he reported seem to us not to be beyond the limits of the accuracy of the method.

We have studied the blood proteins in ten diuretic experiments, in which we have used aminophyllin, mercupurin and mersalyl and have not been able to confirm Shally's findings in regard to shifts in the blood proteins. We have also determined the dried weight of the serum and have not been able to demonstrate any significant or consistent changes that would indicate an effective hydremia.

THE SODIUM AND CHLORIDE RATIONS

Hitzenberger and Engelmann[6] found that the excretion of many inorganic and organic substances, but particularly NaCl, was augmented by mersalyl. Simmert[7] reported similar increases in the urinary NaCl, determined as Cl, with concomitant rises in the blood plasma NaCl, also determined as Cl, occurring in diuresis along with decrease in the hemoglobin and red blood cells. He concluded that his findings indicated a mobilization of the tissue NaCl and a flow of the tissue edema fluid into the blood. Bua[8] on the basis of studies of the effect of mersalyl in diabetes insipidus concluded that the presence of the chloride ion was the sine qua non requirement for the diuretic action of mercury salts. Weingarten[9] of Volhard's school has held, as indisputable, the hydropigenous property of the chloride ion.

Presser and Stahl,[10] however, incriminated the Na ion as the edema producer which effect could be compensated for by a balanced combination with others, as K, Ca, and Mg ions. Bruman and Jenny[11] found a parallelism between sodium excretion and the volume of the urine.

Siedek and Zuckerkandl[12] found more retention of Na than of Cl, therefore Na/Cl$>$ I in pleural and ascitic effusions and in the edema fluid of congestive cardiac failure. Upon the application of diuretics a marked excess of Na over Cl appeared in the urine.

OUR SODIUM AND CHLORIDE DATA

In our attempt to elicit the initiating factors and those at work during the first five to six hours of diuresis, we determined the urinary Na/Cl ratios before, hourly during, and as a follow-up, each twenty-four hours for several days after each of our 25 diuretic experiments with aminophyllin, mercupurin, and

mersalyl. The blood Na remained fixed, but the urinary Na conspicuously increased but usually not in the tremendous proportions of the Cl excretion, so that the ratios Na/Cl as a rule decreased. Occasionally, however, the reverse was noted and the Na/Cl ratio increased. In these studies of ours, neither the mercurials nor the xanthines showed consistent or characteristic effects on the Na/Cl ratio.

If the volume of urine, with its contained sodium and chloride, passed during the control hours, be subtracted from the corresponding figures after injection of the diuretic, we obtain a fair approximation of the excess of these substances mobilized from the tissues during diuresis. By assuming the edema fluid to be an ultrafiltrate of blood plasma, we can calculate the excess amount of sodium and chloride that should be excreted, with the excess urine excretion of the same composition as the tissue fluid. The values for sodium calculated on this basis show a good agreement with those actually found; while the actual excretion of chloride is in most instances greatly in excess of the calculated values.

CREATININE CLEARANCE STUDIES

Four years ago we reported our studies[13] anent the mechanism by which diuresis is accomplished following the intravenous injection of theophylline, as aminophyllin and organic mercurial salts, as salyrgan and combinations of the two in patients with congestive heart failure and edema. We employed the creatinine clearance method as described by Rehberg[14] to measure the glomerular filtration, and we found in our edematous cardiacs, as Schmitz[15] did in the creatinine clearance experiments in the dog, that the xanthines or purines, theophylline ethylene diamine (thephyldine, metaphyllin or aminophyllin), greatly augmented the glomerular filtration, while the mercurial, mersalyl, apparently produced a diuresis by inhibiting tubular reabsorption.

Our clinical experiences along with our experimental theoretical considerations convinced us that there was a very definite complementary action of the xanthines and mercurials when administered in close succession or simultaneously. The clinical results seemed further to support our conception of the mechanism of diuresis and the different points of action of the two types of drugs. We reported further studies using d-xylose as well as creatinine clearance as a measure of glomerular filtration and secured confirmatory evidence of our thesis. Sucrose intravenously was found to have in itself too powerful a diuretic action. White and Monaghan[16] had found the creatinine clearance as good a test as any of glomerular function, and in spite of Shannon's[17] and Fulton's[18] condemnation, we consider that the creatinine clearance in comparative studies, such as we have been doing, has proved satisfactory.

A translation has recently been furnished us of the paper written in Hungarian by Issekutz and Vegh[19] on experimental studies of diuretics on the toxicity and action of various organic mercury compounds, prepared by Foldy and his associates of the Chinion laboratory. In studying the toxicity of the complex sodium acetate salts of mercury, they added a slightly acid theophyllin solution to reduce the strongly alkaline reaction that caused so much pain on

administration of the mercurial. Theophyllin not only produced the neutral reaction but also definitely increased the diuretic action of the mercurial compound in experimental animals.

A new preparation described by the distributors as a sodium salt of camphoric acid, allylamine-methoxy-mercury acetate with theophyllin 3.5 per cent combined, 1.5 per cent free, containing 41.1 per cent mercury, but according

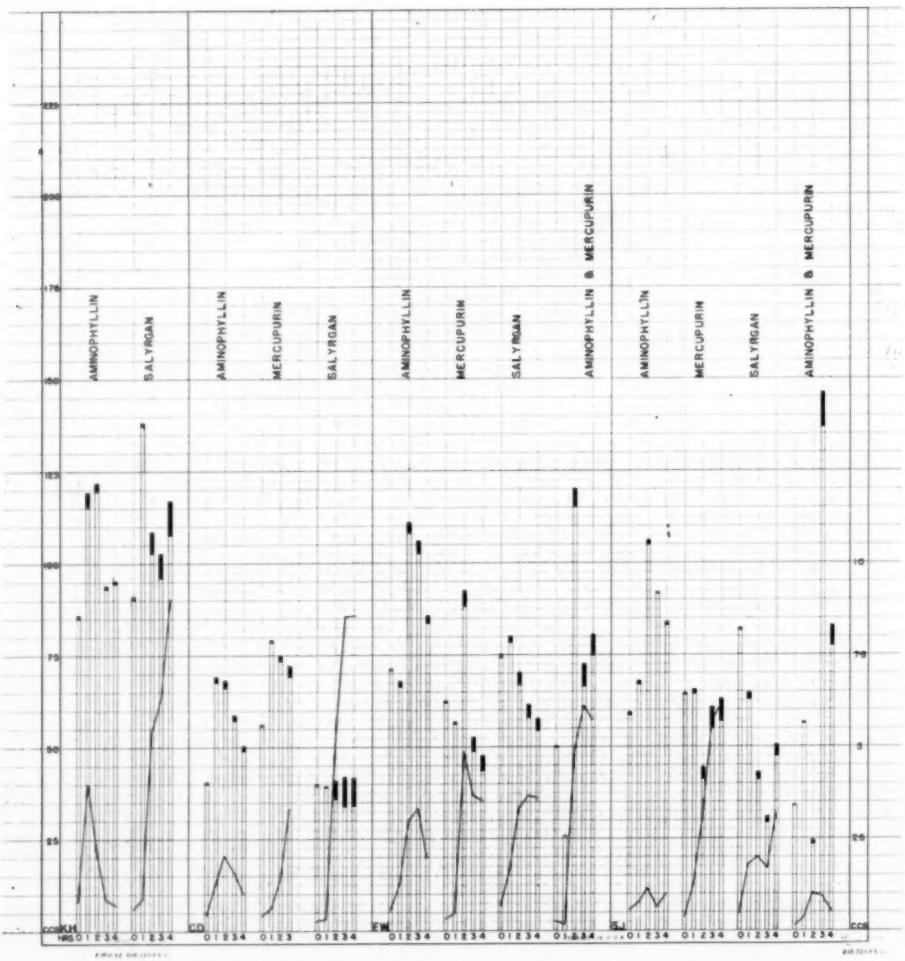

Fig. 1.—Chart showing by hourly columns the height of filtration (top of each column) and the height of reabsorption (bottom of black cap) in cubic centimeters per minute as determined by the creatinine clearance method of Rehberg. The height of the black cap and the rise of the solid line running through the hourly columns indicate the grade of diuresis. Each group of columns represents an experiment following the introduction of one of the diuretics. The experiments on each individual are grouped between the bordering lines and the diuretic used in each experiment is printed above the columns.

to Issekutz and Vegh as containing 38.8 per cent mercury all of which is combined, was introduced into clinical therapeutics. DeGraff, Nadler, and Batterman[20] recently stated that the 13.5 per cent aqueous solution of mercupurin with theophyllin contains 29.1 per cent mercury by weight, so that 1 c.c. contains 0.0393 gm. Hg while mersalyl with 39.5 per cent Hg by weight contains

in 1 c.c. of the 10 per cent solution 0.0396 gm. Hg. Thus the actual difference in mercury content between mersalyl and mercupurin is negligible. Mercupurin differs chemically from mersalyl only in having camphoric acid substituted for the salicylic acid, and theophyllin added. This substitution is said to have made the preparation about half as toxic as mersalyl. The new compound was called for the sake of brevity "novurit" in Europe and in this coun-

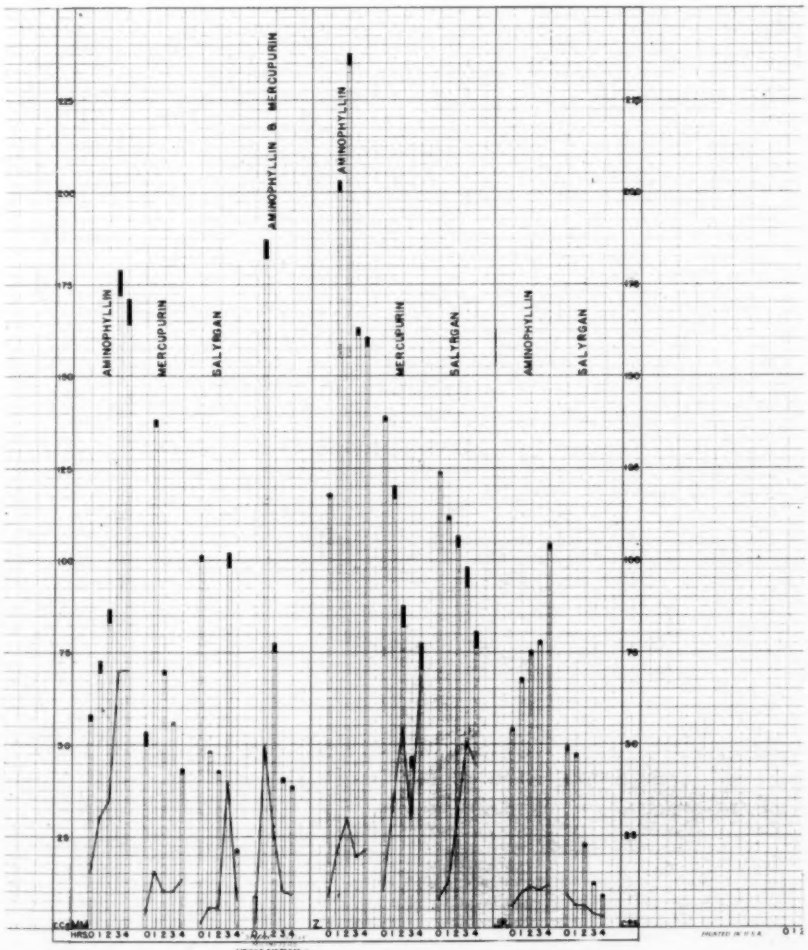

Fig. 2.—Charts on other patients made up just as those in Fig. 1 showing the increased filtration as a rule following aminophyllin intravenously and decreased reabsorption diuresis after mersalyl injection and combination effects with the mercupurin administration.

try "mercupurin." The mercurate camphoric acid without the theophyllin added has been offered as mercurial diuretic suppository "mercurin" for rectal administration.

PRESENT STUDIES ON THE MECHANISM OF DIURESIS

Introduction of the new compound that is a combination of the mercurial and xanthines with reputed superior diuretic properties seemed to confirm our earlier contentions of the different mechanism of action for each of the two

types of diuretics and to lend support to our observations of the complementary action of the two drugs. The claims for the new preparation revived our interest in the subject of the mechanism of diuresis, and we repeated our diuretic studies in edematous heart failure cases following the intravenous injection of mercupurin as well as mersalyl and aminophyllin.

METHOD

The patients were kept at absolute rest in bed on low protein (50 gm.), salt poor (2 gr.), low fat (115 gm.), and high carbohydrate (200 gm.), total calories 2,035, diet and fluid allowance of 1,600 c.c. a day and no drugs other than morphine for the first five days. During this time the renal function studies were done, the blood and urine chemistry examinations were made and accurate charting of the daily twenty-four-hour intake and output of the fluid and body weight of the patient was begun. All twenty-four-hour specimens of urine were sent to our laboratory for complete study.

On the morning of the sixth day the patient was given 10 gm. of creatinine in 100 c.c. of water at 6 A.M. At 7 A.M. a catheter was introduced and the bladder was completely emptied and the urine collected; a control blood specimen was taken at the same time. At 8 A.M. the first-hour urine specimen was removed by catheter and the blood specimen collected. The second control blood and catheterized urine specimens were taken at 9 A.M. and the diuretic aminophyllin in a dose of 0.48 gm. in 10 c.c. of saline, or 2 c.c. of a 10 per cent solution of mersalyl, or 2 c.c. of a 13.5 per cent solution of mercupurin, or the latter fortified with 0.24 gm. of aminophyllin was injected intravenously. After this the blood and catheterized urine specimens were taken every hour for four hours. Increase in urinary flow was consistently observed in the second half hour after injection of mersalyl and in the second hour after the injection of aminophyllin.

Seven patients, with congestive heart failure of varying etiology in each case, were selected and subjected to twenty-five carefully controlled creatinine clearance diuretic studies.

The theophyllin preparation, aminophyllin, was usually given in the first experiment. Occasionally, however, the mercurial, mersalyl, diuretic was administered first. Usually three to five or more days later, or when the body weight returned to the previous level and the urinary output was low and fluctuated very little, the second preparation, the one that had not been used previously, or mercupurin, the new preparation, was introduced intravenously as the diuretic drug. After another interval of at least three days, the one of the three preparations that had not previously been used was administered and its effects were noted. In three instances the mercupurin was fortified with an additional 0.24 gm. in 1 c.c. of aminophyllin and then injected and the more spectacular results were observed and studied.

Evidence of conspicuous smooth muscle relaxing effects of aminophyllin has been repeatedly noted clinically by us in the prompt subsidence of Cheyne-Stokes breathing, the control of paroxysmal nocturnal dyspnea, and particu-

larly in the relief of persistent epinephrine-fast asthmatic states. In some 50 asthmatic patients, intravenous aminophyllin can be shown to result in prompt increase in the vital capacity and blood flow through the lungs. Mercurials do not produce such spectacular and prompt respiratory relief, but after profuse mersalyl diuresis, dyspnea is usually less paroxysmal attacks subside.

THE RESULTS

The present creatinine clearance studies confirmed and supplemented our previous observations. A survey of the data revealed the fact that in the twenty-five studies on the seven edematous patients it seemed to make little difference which drug was introduced first. Of course, the interval of three to five or more days allowed the effect to be dissipated before the second drug was used. In all experiments satisfactory diuresis was accomplished.

The 7 experiments in which aminophyllin from an ampule of 2 c.c. containing 0.48 gm. diluted 10 times with saline was used intravenously presented further conclusive evidence of a conspicuous increase in glomerular filtration in every instance. In the 7 experiments in which mersalyl, 2 c.c. of a 10 per cent solution, was introduced, the creatinine clearance studies showed consistently little if any rise and usually a gradual decrease in the filtration as the diuresis increased. In other words there was, along with the increasing defect in reabsorption, a slight decrease in filtration. In four instances in which 2 c.c. of 13.5 per cent solution of mercupurin with 3.5 per cent combined, 1.5 per cent free theophyllin was used, the creatinine clearance showed a predominance of the mercurial effect in two of the cases, that is, there was no rise in filtration but decreased reabsorption, while the xanthine effect, an increased filtration, predominated in three instances. The increase in filtration was a very definite, though often somewhat delayed, occurrence. In three instances in which mercupurin was fortified with aminophyllin there was often a very temporary decrease in the filtration before diuresis began but this was followed by a conspicuous rise in the glomerular filtration along with the characteristic mercurial impairment of tubular absorption. This fortified combination produced a more striking diuresis.

Blumgart, Gilligan, Levy, Brown and Volk[21] were not able to demonstrate any theophyllin augmentation of filtration in six studies on three normal individuals who were under very carefully controlled conditions.

In our previous and present creatinine clearance studies in edematous patients, we have demonstrated a total of 17 experiments in which theophyllin preparations were used, with a very definite and often conspicuous increase in glomerular filtration. In 3 instances in which aminophyllin was combined with mercupurin, a similar evidence of definite increase in filtration was obtained and likewise in 3 out of the 5 cases in which mercupurin alone was used, a transient increase in filtration was demonstrated. In the 17 experiments in which mersalyl was used and the creatinine clearance studies carried out, a defect in reabsorption was the striking finding in every instance. In the 7 mersalyl experiments of the present series and in 2 of 5 mercupurin experi-

ments, there were not significant increases in glomerular filtration; in fact, there was an actual decrease in filtration as well as a conspicuous decrease in reabsorption that accounted for the diuretic effect.

NEW PREPARATIONS

The creatinine clearance studies following the introduction intravenously of the new preparations are indirectly confirmatory. In comparative studies in the same individuals, glomerular filtration figures midway between those obtained with mersalyl alone on the one hand and those following aminophyllin alone on the other hand, have been repeatedly obtained. In only one mercupurin experiment was the increase in filtration at all conspicuous, while 2 of the other 4 showed slight rises in filtration. The inhibitive mercurial effect on reabsorption was, of course, always noted. When the mercupurin was fortified with 1 c.c. of aminophyllin, an unexplained very temporary decrease in filtration was regularly recorded before clinical diuresis had started. This was noted in 2 of 3 instances but all showed within the next hour a very striking increase in filtration as well as the defective reabsorption.

These data not only supported the conception of a difference of the renal site or mode of action of diuretics, but also supported our contention of a dual action of the combined mercurial salt and theophyllin. The experiments seem to furnish evidence that the theophyllin constituent of the new preparation does more than merely neutralize the excessive alkalinity of the mercury salt and actually in these acute experiments it seemed to augment the diuretic property of the latter. The more striking diuresis that resulted from the mercupurin fortified with the additional theophyllin in the form of 0.24 gm. in 1 c.c. of aminophyllin suggests that more theophyllin might be added to mercupurin to advantage.

THE NEWER DIURETIC COMPOUNDS IN CLINICAL PRACTICE

This combination of organic mercurials and theophyllin was introduced into clinical practice in Continental Europe as "novurit," and at least a dozen clinical reports bear witness to its efficiency and its nontoxicity. Hahn,[22] Popper,[22] and Saxl[22] are among those who have reported favorably on the preparation. In this country Crawford and McDaniel[23] studied the effect of mercupurin in 15 patients, 10 with edema of heart failure, and 5 with ascites of hepatic cirrhosis, all in advanced stages after long periods of invalidism. Their results were uniformly very satisfactory and the 20 injections of mersalyl used as control gave definitely less diuretic response than did the 118 injections of mercupurin. Studies of the postdiuretic urine specimens showed no renal irritation, and blood urea levels did not rise after the injection of either preparation. No untoward reactions from the injections of either drug were encountered. Steuer and Wolpaw[24] reported a very small series of 35 injections of mercupurin with no control of mersalyl injections and came to conclusions which added nothing to those previously made on more substantial bases. In England, Parkinson and Thomson[25] have conducted studies of a clinical com-

parative nature both with mercupurin and mersalyl intravenously and with mercurin administered as suppositories. They found the newer preparations to be rather more efficient.

The most complete clinical studies of mercupurin were made by DeGraff, Nadler, and Batterman. Their 20 edematous patients were observed under carefully controlled conditions and the body weight fluctuations taken as the evidence of effectiveness of diuretic agent. Besides studying the effect of mersalyl and mercupurin alternately in the same patient, these investigators secured a preparation containing only the organic mercurial or mercupurin without the theophyllin. Contrast of the effect of this pure mercurial preparation used intravenously and under the same conditions with the effects of the combination, mercurial + xanthine, preparations showed that the theophyllin added definitely to the effectiveness of mercupurin. They had no general toxic mercurial and no local reactions from intravenous administration of nearly 100 injections. They concluded that mercupurin was a safe diuretic as well as an effective one and that the combination preparation induces a greater diuresis than mercurials alone and that the superiority of the xanthine mercurial is chiefly if not entirely due to the presence of the theophyllin.

OUR CLINICAL STUDIES

Comparative clinical studies of mercupurin diuresis as compared with mersalyl diuresis have been carried out in the wards of the John Sealy Hospital for the past two years, and during the past year mercurin suppositories containing mercamphyl, a mercurial salt similar to mersalyl with camphoric acid instead of salicylic and without theophyllin and put up in a cocoa butter, have been under clinical investigation. Our data concern results in 70 cases of patients with edema who have been observed under standard conditions of a preliminary three- to five-day rest in bed, on a constant intake of 1,600 c.c. of fluid and a low protein salt poor diet. Only 5 of these patients suffered primarily from the effects of cirrhosis of the liver, the other patients presented evidences of organic heart disease of one type or another with congestive failure in various stages and of varying degrees.

In these 70 patients, 200 injections of mercupurin were given intravenously and alternately, for comparison, 115 injections of mersalyl were given in 2 c.c. doses of each, and 64 mercamphyl (mercurin) suppositories were administered. The order of the use of the drugs varied from time to time and the initial drug used was alternated. Sometimes the preliminary administration of acid salts simultaneously administered and other times omitted was employed and definite augmentation was always noted no matter what preparation was used. In 25 of the suppository cases for experiment, the preparatory enema was omitted and the results were so poor that they should not be included in the average, and in the other half of the cases an enema was given.

The diuretic results of all of these injections and suppositories were tabulated; the percentage of increase in urinary output during the first and second and third twenty-four-hour periods following the administration of the drug as contrasted to the control daily outputs were calculated and summarized for

us by C. C. Pearson, a senior, and R. C. Douglas, a junior medical student, employed on NYA. funds. Our clinical results are given in Table I.

TABLE I

CLINICAL SUMMARY OF DIURETIC EFFECTS

NO. OF INJECTIONS	DRUG	AVERAGE INCREASE IN OUTPUT OF URINE			
		1ST 24 HR.	2ND 24 HR.	3RD 24 HR.	TOTAL
200	Mercupurin	430%	92%	91%	613%
115	Mersalyl	293	92	42	427
25	Mercurin suppositories without enema	92	41	95	228
70	Mercurin suppositories with enema	264	66	25	355

Taking all of the results as they come, the conditions of course varying, now in favor of one drug and now in favor of the other, seemed to favor mercupurin. The average responses for the first, second, and third days following administration of mercupurin showed increases of 430 per cent, 92 per cent, and 91 per cent, respectively, a total of 613 per cent over the urine output of the day preceding the injections; while for mersalyl the averages during the same period showed 293 per cent, 92 per cent, and 42 per cent, a total of 427 per cent increase for the three days over the control day. A secondary rise after the first day was occasionally noted following mercupurin. The level of the control day was, of course, established at a fairly fixed level by the conditions of the experiment, namely, the three- to five-day rest preceding the administration. The table gives a summary of the clinical diuretic effects and also shows that both mercurial diuretics accomplish maximal effect within the first day; both drop about equally during the second day; but there was often persistence of the effects during the third day after mercupurin, while the mersalyl diuretic effect dropped considerably on the third day.

The mercurin suppositories showed a 264 per cent increase for the first day, 66 per cent during the second, and 25 per cent during the third day, a total of 355 per cent following their use; a definitely better effect, in fact, a threefold augmentation of urinary output when the enema was used to prepare the bowel. The diuretic effects did not persist as actively as after intravenous use, showing 67 per cent on the second day and 73 per cent on the third, a total of 370 per cent. Preliminary oral administration of xanthines and of acid salts likewise distinctly augmented the diuresis that resulted from mercurin suppositories.

In only one instance have we had any evidence of renal irritation, and in this case the irritation was first noted after the introduction of mersalyl but did not clear up under continued use of mercupurin. The albuminuria, however, cleared after a rest period, and the subsequent administration of mercupurin caused a prompt exacerbation of the albuminuria; nitrogen retention, on the other hand, actually decreased under the continued use of mercupurin, even though the albuminuria persisted. No other albuminurias nor any instances of intolerance or toxicity of mercupurin were encountered in our series; in fact, in cases in which stomatitis and colitis had been previously produced by other mercurials, mercupurin was tolerated without such reactions.

Anuria as a result of glomerular tubular destruction which we have noted in two instances following the use of other mercurials intravenously was not encountered in this small series. Mercurin suppositories produced renal irritation in one instance. It must, therefore, be remembered that the suppository contains 0.5 gm. (7.5 gr.) of mercury.

COMMENTS

The results bear out the prediction and the clinical experiences of our earlier studies[13] in which we pointed out the augmentary effect of a combination of theophyllin and the mercurials.[13] Our clinical findings with mercupurin further confirm those of other investigators. It is evident that the combination preparation of xanthine and mercurial has advantages in the matter of diuretic efficiency.

Our repeated carefully controlled and comparative creatinine clearance studies in seven patients of this series corroborate our previous contentions; namely, that xanthine preparations, particularly theophyllin or aminophyllin, definitely increase the minute glomerular filtration, while the pure mercurial, as mersalyl, acts quite otherwise in most instances, actually decreasing the glomerular filtration and producing a diuresis primarily by inhibiting tubular reabsorption.

The mercupurin creatinine clearance experiments, five in number, carried out in the same way on the same patients, sometimes preceding those with the other preparations and sometimes between them and sometimes afterward, showed in 2 instances clearance figures more like those of mercurial action, namely, decrease in tubular reabsorption, while in the 3 others the xanthine effect of increased glomerular filtration was also temporarily evident.

The creatinine clearance studies in three instances of mercupurin fortified with 0.024 gm. of aminophyllin showed the most striking combination effect of distinctly increased filtration as well as decreased reabsorption. These experiments in confirming all of our previous studies furthermore seem to establish definitely that the theophyllin does not merely serve to neutralize the excess alkalinity of the mercurial salt but seems to augment the diuresis. The experiment with mercupurin fortified by additional theophyllin suggests that more of the xanthine could be added to the mercupurin to advantage. The theophyllin simultaneously used or ammonium chloride or nitrate by mouth along with the pure mercurial suppository would be rational therapeutics.

CONCLUSIONS

1. We believe that further evidence is added that diuretics, both the xanthine and mercurial, act directly upon the kidney.

2. It is apparent from these creatinine clearance studies that the two types of diuretics act somewhat differently and on different parts of the renal unit. The xanthines affect primarily the glomerular tuft of the unit and by increasing the flow of blood increase filtration, while the mercurial apparently acts by changing the tubular epithelium, so that it is less permeable to the filtrate of water and salts and thus by decreasing reabsorption accomplishes increased urinary output.

3. The combination of xanthine with mercurial seems to be logical on theoretical grounds, as we have previously pointed out. The fact that the theophyllin with its mild acidity helps to neutralize the excessive alkalinity of the mercurial salt is another advantage that makes for safety and ease of tolerance.

4. The clinical experiments seem to corroborate the theoretical considerations, in that the combination preparations have definite advantages in efficiency when contrasted to the mercurials in pure form and over aminophyllin when introduced alone.

5. The mercury suppository, mercurin, constitutes an innovation that has many advantages, even though the results are not as spectacular as those following intravenous injections of mercupurin or mersalyl; nevertheless, they are quite striking and gratifying. Even if the preparation were only half as active, it would still have a place for itself by virtue of the ease of administration, and the comparative safety for the rectal administration in contrast to its intravenous medication. In fact, the latter mode of therapeutic approach is never entirely without danger, and whenever effective, other routes of administration should be chosen. This is particularly so for the man in the field, the general practitioner, who is not regularly doing intravenous work. It is quite an extra job to prepare and transport sterile equipment necessary for intravenous injections.

REFERENCES

1. Meyer, H. H., and Gottlieb, R.: Die Experimentelle Pharmakologie, Berlin, 1933, Urban and Schwarzenberg, 489.
2. Jackson, D. E.: The Pharmacologic Action of Mercury in Organic Combination, J. Pharmacol. & Exper. Therap. **29**: 471, 1926.
3. Richards, A. N.: The Nature and Mode of Regulation of Glomerular Function, Am. J. M. Sc. **170**: 781, 1925.
4. Shally, H. O.: Veränderungen der Bluteiweisskörper bei der Salyrgandiurese, Deutsches Arch. f. klin. Med. **177**: 368, 1934.
5. Bieter, R. N., and Wright, H. N. G.: Fundamental Studies on the Pharmacology of Mercury Diuretics. In The Kidney in Health and Disease, Berglund, et al., Philadelphia, 1935, Lea & Febiger, p. 701.
6. Hitzenberger, K., and Engelmann, F. L.: Ueber das spezifische Gewicht des Harnes bei Salyrgandiurese, Ztschr. f. klin. Med. **129**: 290, 1935.
7. Simmert, H. U.: Über das diuretische wirkung organischer Quecksilberverbindungen, Klin. Wchnschr. **14**: 530, 1935.
8. Bua, F.: Über die Wirkungsart der Quecksilberdiurese bzw- anti-diurese, Klin. Wchnschr. **14**: 934, 1935.
9. Weingarten, R.: Über Kochsalzersatzmittel, München. med. Wchnschr. **79**: 965, 1932 I.
10. Presser, H., and Stahl, R.: Untersuchungen ueber hydropigene Ioninwirkungen auf lebende Gewebe mittels Durchströmung in Tierversuch, München. med. Wchnschr. **80**: 559, 1933 I.
11. Bruman, F., and Jenny, F.: Der Einfluss der Ernährung auf den Stoffunisatz bei der Arbeit, Deutsches Arch. f. klin. Med. **177**: 527, 1934.
12. Siedek, H., and Zuckerkandl, F.: Die Bedeutung des Natrium—Chlorquotienten im Harn beim "Volhardschen" Wasserversuch, Klin. Wchnschr. **14**: 1428, 1935.
13. Herrmann, G., Stone, C. T., and Schwab, E. H.: Some Studies in the Mechanism of Diuresis in Patients With Congestive Heart Failure, Tr. A. Am. Physicians **47**: 279, 1932.

    Herrmann, G., Stone, C. T., Schwab, E. H., and Bondurant, W. W.: Diuresis in Patients With Congestive Heart Failure, J. A. M. A. **99**: 1647, 1932.

    Herrmann, G., Schwab, E. H., and Stone, C. T.: Further Studies on the Mechanism of Diuresis in Patients With Congestive Heart Failure, Tr. A. Am. Physicians **48**: 364, 1933.

    Herrmann, G., Schwab, E. H., Stone, C. T., and Marr, W. L.: On the Advantage of Alternating the Vegetable and Metallic Diuretics in the Treatment of Edema of Congestive Heart Failure, J. Lab. & Clin. Med. **18**: 902, 1933.

Schwab, E. H., Herrmann, G., and Stone, C. T.: The Complementary Action of Certain Antiedemic Drugs, Texas State J. Med. **29**: 240, 1933.

Stone, C. T., Herrmann, G., and Schwab, E. H.: The Treatment of Edema in Congestive Heart Failure, South. M. J. **27**: 113, 1934.

14. Rehberg, P. B.: Studies on Kidney Function. I. The Rate of Filtration and Reabsorption in the Human Kidney, Biochem. J. **20**: 447, 1926.
15. Schmitz, H. L.: Studies on the Action of Diuretics. I. The Effect of Euphyllin and Salyrgan Upon Glomerular Filtration and Tubular Reabsorption, J. Clin. Investigation **11**: 1075, 1932.
16. White, H. L., and Monaghan, B.: A Comparison of the Clearances of Creatinine and of Various Sugars, Am. J. Physiol. **106**: 16, 1933.
17. Shannon, J. A.: The Renal Excretion of Creatinine in Man, J. Clin. Investigation **14**: 403, 1935.
18. Davenport, L. F., Fulton, M. N., van Auken, H. A., and Parsons, R. H.: Creatinine Clearance as Measure of Glomerular Filtration in Dogs, With Particular Reference to Effect of Diuretic Drugs, Am. J. Physiol. **108**: 99, 1934.
19. Issekutz, B., and Vegh, F.: A szerves higanyvegyuletek diuretikus hatasarol, Orvosi hetil. **45**: 1928.
20. DeGraff, A. C., Nadler, J. E., and Batterman, R. C.: A Study of the Diuretic Effect of Mercupurin in Man, Am. J. M. Sc. **194**: 526, 1936.
21. Blumgart, H. L., Gilligan, D. R., Levy, R. C., Brown, M. G., and Volk, M. C.: Action of Diuretic Drugs. I. Action of Diuretics in Normal Persons, Arch. Int. Med. **54**: 40, 1934.
22. Hahn, A.: Novurit, ein neues Diuretikum, Wien. klin. Wchnschr. **42**: 1477, 1929.

Popper, L.: Ein neues Quecksilber—Diuretikum, Novurit, Med. Klin. **25**: 912, 1929.

Popper, L.: Further Experiences With the Mercurial Diuretic, Novurit, Med. Klin. **26**: 1229, 1930.

Saxl, P.: Fortschritte der Diuresetherapie, Wien. klin. Wchnschr. **43**: 916, 1930.

23. Crawford, J. H., and McDaniel, W. S.: Some Observations on Mercurial Diuretics, Ann. Int. Med. **8**: 1266, 1935.
24. Steuer, L. G., and Wolpaw, S. E.: The Diuretic Action of Mercupurin, J. Lab. & Clin. Med. **21**: 298, 1935.
25. Parkinson, J., and Thomson, W. A. R.: A Mercurial (Mercurin) Suppository as a Diuretic for Cardiac Edema, Lancet **1**: 16, 1936.

A STUDY OF SERUM CHOLESTEROL IN PATIENTS WITH PEPTIC ULCER*

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IN REVIEWING the literature, we have been unable to find any record of free and total cholesterol determinations in the blood serum of patients with peptic ulcer. We are therefore reporting a series of such determinations, with the hope that the results may be of some significance in further understanding the pathogenesis of this condition.

The study was carried out on patients either admitted to the medical and surgical wards of the Presbyterian Hospital, or under observation in the Vanderbilt Clinic. No case is included in which confirmation of the clinical diagnosis of peptic ulcer was not obtained by x-ray study after a barium meal (deformity of the mucosa, or visible crater).

This series included a total of 59 patients, of whom 7 were on the surgical services, 9 ambulatory to the clinic, and 33 on the medical services. The total age range of these patients, of whom 45 were male and 14 female, was from twenty-two to seventy-one years, but 46 patients fell between thirty and fifty years of age. Seven of these patients had a gastric ulcer, while 52 had a duodenal ulcer.

Samples of blood were obtained by venipuncture from the patients who had been fasting for a minimum of ten hours. In every instance the blood was obtained as soon as possible after the patient was admitted to the wards or clinic, in order to reduce as far as possible the length of time that the patients were on a high fat diet. As part of our procedure for all groups of determinations, the specimens were collected in 15 c.c. tubes and placed in the refrigerator within an hour, the tubes being tightly stoppered. Twenty-four hours later the clot was rimmed with a thin glass rod, and the samples centrifuged for fifteen minutes at 1,500 r.p.m. The serum was pipetted into tubes and recentrifuged for five minutes to insure removal of cells. The determination of free and total cholesterol in the serum was carried out by the method of Schoenheimer and Sperry,[1] with the use of the colorimeter as described by Fitz[2] and the ink standard originated by Shapiro and others.[3]

The statistical procedures employed are as follows:

1. Standard Deviation of a Single Observation (σ) $= \sqrt{\frac{\Sigma d^2}{N}}$

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Received for publication, July 29, 1936.

2. Probable Error of the Mean (P. E. m.) $= .6745 \times (\sigma \diagdown \sqrt{N})$

3. Significance Value (S) $= \dfrac{\text{Difference of Means}}{\sigma \text{ d of Diff.}}$

4. Standard Deviation of a Difference Between Means $(\sigma \text{ d}) = \sqrt{\left(\frac{\sigma_1^2}{N_1}\right) + \left(\frac{\sigma_2^2}{N_2}\right)}$

d, Algebraic difference between arithmetical mean and a single observation.
d^2, Summation of differences^2 for a series.
N, Number of determinations.

FINDINGS

Fig. 1 shows a frequency distribution curve for the free and total cholesterol concentrations. From this curve may be seen the complete range of findings, which are 32.1 to 83.7 mg. per cent and 98.1 to 300.0 mg. per cent for free and total cholesterol, respectively. The total range of the per cent of free in total cholesterol is from 24.4 to 38.6 per cent.

Applying the statistical formulas noted above, the mean values and standard deviations for each of the three groups are obtained (Table I). The mean for the total cholesterol is 160.8 mg. per cent ± 38.7 mg. per cent (S.D.). This indicates that at least two-thirds of all values here fall between 122.1 mg. per cent and 195.5 mg. per cent. In like fashion the mean for the free cholesterol

TABLE I

FREE, TOTAL, AND PERCENTAGE OF FREE IN TOTAL CHOLESTEROL VALUES FOR PEPTIC ULCER PATIENTS

TOTAL CHOLESTEROL	COMPLETE RANGE OF VALUES IN MG. %	ARITH. MEAN MG. %	STANDARD DEVIATION (σ)	PROBABLE ERROR OF MEAN	SIGNIFICANCE VALUE (S) COMPARED WITH NORMAL
	98.1----300.0	160.8	38.7	3.4	8.4
Free cholesterol	32.1-----83.7	47.7	11.1	0.97	
Per cent free in total cholesterol	26.3%---34.5%	30.4	4.1%	0.35	

determinations is 47.7 mg. per cent ± 11.1 mg. per cent (S.D.), and that for percentage of free cholesterol is 30.4 per cent ± 4.1 per cent (S.D.).

In comparing the findings of our peptic ulcer series with normal values, it was deemed most consistent to employ the findings of W. M. Sperry,[4] who kindly made available his figures before actual publication, inasmuch as the basic procedure for both groups of determinations was that of Schoenheimer and Sperry.[1] In a series of 126 determinations on normal adults, male and female, he obtained a mean total cholesterol concentration of 209.8 mg. per cent ± 48.6 mg. per cent. There was no significant difference obtained between the pre- and postabsorptive divisions of this series, remarkable constancy being maintained for the percentage free cholesterol, the mean value ascertained as 26.9 per cent ± 1.4 per cent. Fig. 2 is a frequency distribution curve for the total cholesterol concentrations obtained by Sperry, the complete range being from 131.5 mg. per cent to 392.0 mg. per cent.

The most outstanding difference between our series and such a group of normals is a definite shift to the left (lower values) of the frequency distribu-

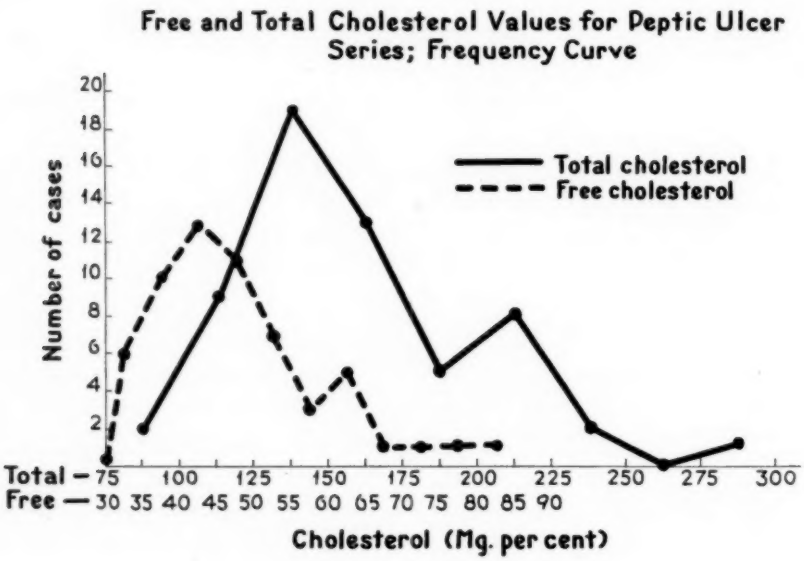
Free and Total Cholesterol Values for Peptic Ulcer Series; Frequency Curve
Number of cases
20
18
16
14
12
10
8
6
4
2
Total cholesterol
Free cholesterol
Total — 75 100 125 150 175 200 225 250 275 300
Free — 30 35 40 45 50 55 60 65 70 75 80 85 90
Cholesterol (Mg. per cent)

Fig. 1.

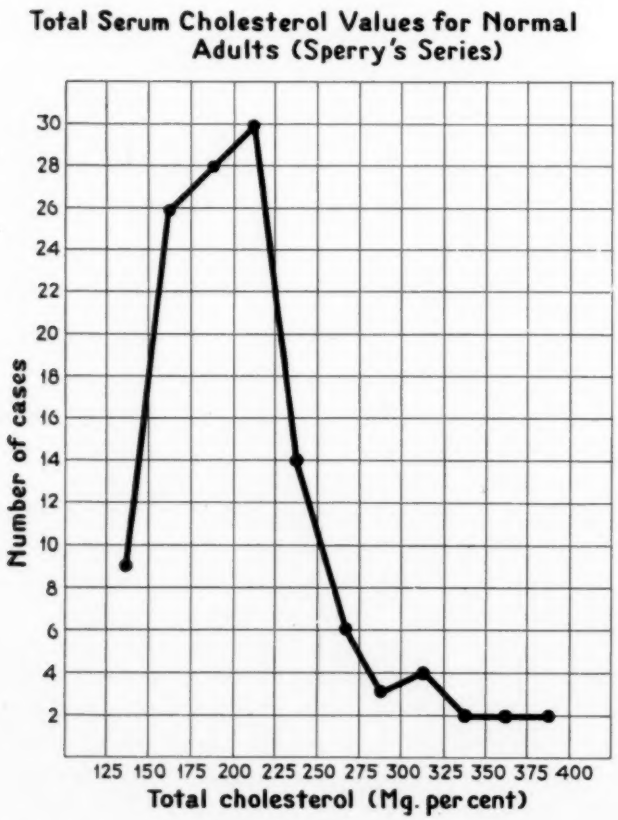
Total Serum Cholesterol Values for Normal Adults (Sperry's Series)
Number of cases
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
125 150 175 200 225 250 275 300 325 350 375 400
Total cholesterol (Mg. per cent)

Fig. 2.

tion curve for total cholesterol, as may be seen from Figs. 1 and 2. The significance value (S) is obtained between our mean of 160.8 mg. per cent ± 38.7 mg. per cent and Sperry's normal mean of 209.8 mg. per cent ± 48.6 mg. per cent, and this is found to be 8.4, thereby establishing the trend toward a hypocholesteremia for our series of patients with peptic ulcer. The other observation of importance is the generally higher percentage free cholesterol found in the peptic ulcer patients. Although not marked, this change from the normal suggests that the fall in total cholesterol concentrations is due in greater measure to the decrease of ester cholesterol rather than free (or uncombined) cholesterol.

It is of interest to note that the range of values for our peptic ulcer series, duplicates in extent, even though shifted to slightly lower values, the findings in the series of so-called normal individuals. We have been unable to find factors in individual cases which would consistently account for the values obtained therein. Particular attention was paid to the question of anemia, but no correlation could be obtained between cholesterol concentration and level of erythrocytes or hemoglobin. Table II gives a brief summary of findings in each case used for this study.

DISCUSSION

The true significance of the tendency to hypocholesteremia in patients with peptic ulcer cannot be stated without further investigation. Cantarow,[5] in his review of cholesterol knowledge, finds that hypocholesteremia occurs in hyperthyroidism, acute infections, pernicious and aplastic anemias, hemolytic jaundice, tuberculosis, widespread liver damage, prostatic and intestinal obstruction. Essentially, these conclusions are concurred with by Stone[6] in his review. It is beyond the scope of this paper to assign any of the above factors as the etiologic factors in peptic ulcer, on the basis of our cholesterol findings. It is of interest to note, however, that Sperry[4] found an elevated percentage-free cholesterol only in cases of infection or liver damage, and that we[7] demonstrated a hypocholesteremia in acute and subacute stages of infection (primary and secondary lueties). Stoesser[8] has likewise found a comparable hypocholesteremia in acute infections with an elevation of the percentage-free cholesterol at the height of the infection.

The hypocholesteremic trend in this series of patients with peptic ulcer is doubly significant, since almost all of these individuals had been on a relatively high fat diet for varying periods of time. One cannot but feel that the tendency to lowered blood cholesterol values here argues against the cholesterol level standing in relationship to exogenous food metabolism. Agreeing with this point of view is a partially completed series of determinations on medical students in which we[9] found no significant variations in either free or total serum cholesterol. The work of Blotner[10] and Boyd[11] is in accord with these findings. In contrast to the above, however, it is only fair to point out the studies of Okey and Stewart[12] and the review of Cantarow[5] who feel that, while there are no true diurnal cholesterol fluctuations, the blood levels will be higher in individuals on a high fat diet for long periods of time.

TABLE II

ANALYSIS OF CASES

NO.	AGE	NUTRITION	DURATION SYMPTOMS	ANEMIA	ADDITIONAL REMARKS	CHOLESTEROL		
						TOTAL MG. %	FREE MG. %	% FREE
1	45	Poor	7 mo.	None	Osteoarthritis Gastroenterostomy	102.3	34.5	33.4
2	22	Good	6 yr.	Moderate	None	124.0	48.0	38.6
3	29	Fair	5 yr.	None	None	149.0	46.6	31.3
4	45	Poor	7 yr.	Severe	Perf. and gastro-enterostomy	144.8	36.8	25.1
5	71	Poor	1 yr.	Moderate	None	226.0	83.7	35.5
6	47	Excell.	12 yr.	Moderate	None	195.9	51.9	26.5
7	40	Fair	8 yr.	None	Lost 10 pounds, 6 mo.	220.0	53.3	24.2
8	50	Fair	6 yr.	None	Pyloric constric.	200.0	64.6	32.3
9	46	Good	10 yr.	None	None	204.8	65.3	30.8
10	40	Fair	6 yr.	None	Hypertensive cardiac disease	156.8	42.1	26.8
11	36	Good	7 yr.	Severe	Transfused 4 days prior to	154.8	42.5	27.4
12	47	Excell.	24 yr.	None	None	139.7	46.6	39.1
13	37	Fair	12 yr.	None	None	133.6	48.2	35.6
14	44	Good	5 yr.	None	None	185.5	60.2	32.4
15	49	Poor	12 yr.	None	None	201.2	60.1	30.0
16	26	Good	1 mo.	None	None	145.2	46.6	32.3
17	63	Fair	10 yr.	Slight	None	98.1	32.1	32.6
18	49	Fair	5 yr.	None	Syphilis, cured 1925?	127.5	36.8	28.0
19	31	Good	5 yr.	None	Gastroenterostomy 2/36	145.2	44.2	30.4
20	36	Poor	?	None	None	149.0	42.2	28.2
21	34	Poor	2 yr.	Moderate	None	170.6	55.7	32.6
22	31	Poor	9 yr.	None	Spastic colon	151.0	40.4	26.5
23	59	Poor	23 yr.	Marked	Marked cachexia	171.6	45.0	27.0
24	39	Poor	1 mo.	None	None	218.2	64.0	29.4
25	42	Poor	7 yr.	None	None	143.4	37.5	25.7
26	47	Good	3 wk.	Marked	None	130.4	40.1	30.7
27	52	Good	2 yr.	Moderate	None	173.9	53.8	30.9
28	41	Fair	10 yr.	None	None	146.2	39.1	26.5
29	24	Poor	2 yr.	Marked	Hematuria (cause unknown)	121.6	37.1	30.5
30	26	Good	5 days	Moderate	Migraine	147.3	43.4	29.4
31	42	Fair	?	None	None	117.8	38.2	32.4
32	31	Fair	8 yr.	Marked	Syphilis, cured	109.2	34.0	31.3
33	33	Poor	6 wk.	None	None	241.6	61.5	25.3
34	35	Good	6 yr.	None	None	159.6	36.9	23.1
35	50	Poor	11 yr.	Marked	Carcinoma of stomach also	208.0	51.4	24.6
36	39	Good	12 yr.	None	Syphilis, 1924, cured	150.1	41.6	27.7
37	55	Poor	2 yr.	Marked	None	300.0	79.8	26.6
38	39	Good	11 yr.	None	None	139.8	44.3	31.6
39	47	Poor	10 yr.	None	None	147.9	44.8	29.9
40	54	Poor	32 yr.	Marked	Died of pneumonia 4 days after	120.4	39.0	32.6
41	42	Good	2 yr.	None	None	120.1	33.6	28.0
42	56	Poor	26 yr.	None	None	214.2	58.2	28.0
43	25	Good	5 yr.	Marked	None	201.2	52.6	26.2
44	29	Poor	4 yr.	None	None	121.4	34.5	31.3
45	32	Fair	3 mo.	Marked	Cryptorchid (uni-lateral)	166.0	49.9	30.1
46	60	Poor	2 yr.	Moderate	None	138.2	47.4	34.3
47	66	Fair	?	None	Healed T. B.	162.7	53.8	32.6
48	36	Fair	9 yr.	None	None	190.0	58.1	30.5
49	61	Fair	1 yr.	Moderate	Hyper. Cardiac dis. Hypertrophy prostate	140.0	38.3	27.9
50	45	Poor	2 yr.	None	None	168.0	48.0	28.6

TABLE II—CONT'D

NO.	AGE	NUTRITION	DURATION SYMPTOMS	ANEMIA	ADDITIONAL REMARKS	CHOLESTEROL		
						TOTAL MG. %	FREE MG. %	% FREE
51	47	Poor	?	Marked	Undernourished	100.4	34.0	35.4
52	39	Fair	7 yr.	None	None	222.0	73.3	33.1
53	32	Poor	?	None	None	116.0	38.6	33.3
54	31	Poor	20 yr.	None	None	165.2	51.6	31.8
55	55	Good	3 yr.	None	None	152.3	45.6	29.7
56	29	Good	2 yr.	None	None	144.9	48.5	33.3
57	27	Excell.	4 days	Marked	None	136.4	41.4	30.3
58	34	Fair	13 yr.	None	None	176.0	42.4	23.9
59	52	Good	?	Moderate	None	142.2	46.6	32.8

SUMMARY

A study of free and total cholesterol values for the blood serum of patients suffering with peptic ulcer has been presented. A total of 59 patients were used, of whom 45 were males and 14 females.

1. The range of total serum cholesterol values was from 98.1 mg. per cent to 300 mg. per cent, with a mean value of 160.8 ± 38.7 mg. per cent (S.D.).

2. The range of free cholesterol values was from 32.1 mg. per cent to 83.7 mg. per cent with a mean value of 47.7 ± 11.1 mg. per cent.

3. The range of percentage-free cholesterol was from 24.4 per cent to 38.6 per cent with a mean value of 30.4 per cent ± 4.1 per cent.

4. These determinations were made by the Schoenheimer and Sperry technic with modifications as noted above; and were compared with normal values obtained by Sperry, with the following results: There is a significant lowering of the mean value for total cholesterol, with a rise in the percentage of free. indicating the fall in total cholesterol to be due mainly to a fall in esters.

5. Since almost all of these patients were on a high fat diet, the lowered blood cholesterol values tend to favor the theory that these levels are independent of exogenous food metabolism.

We wish to express our indebtedness to Dr. W. M. Sperry of the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, for his untiring interest and aid in this study.

REFERENCES

1. Schoenheimer, R., and Sperry, W. M.: A Micromethod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**: 745, 1934.
2. Fitz, F.: The Application of the Colorimeter to the Schoenheimer Sperry Metnod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **109**: 23, 1935.
3. Shapiro, A., Lerner, H., and Posen, E.: A Fixed Color Standard for Cholesterol Determinations, Proc. Soc. Exper. Biol. & Med. **32**: 1300, 1935.
4. Sperry, W. M.: The Relationship Between Total and Free Cholesterol in Human Blood Serum, J. Biol. Chem. **117**: 341, 1937.
5. Cantarow, A.: Progress in Cholesterol Metabolism, Internat. Clin. **2**: 237, 1935.
6. Stone, A.: Clinical Importance of Cholesterol, Southern M. J. **28**: 705, 1935.
7. Feraru, F., and Offenkrantz, F. M.: A Study of Cholesterol Values in Luetics, Am. J. Syph. Gonorr. & Ven. Dis. **21**: 267, 1937.
8. Stoesser, B.: Cholesterol Studies in Acute Infections, Proc. Soc. Exper. Biol. & Med. **32**: 1324, 1935.
9. Offenkrantz, F. M., and Karshan, M.: A Study of Serum Cholesterol Values in Normal Children, Am. J. Dis. Child. To be published.
10. Blotner, A.: Blood Fat Tolerance Tests in Malnutrition and Obesity, Arch. Int. Med. **55**: 121, 1935.
11. Boyd, E. M.: Diurnal Variations in Plasma Lipids, J. Biol. Chem. **110**: 61, 1935.
12. Okey, R., and Stewart, D.: Diet and Blood Cholesterol in Normal Women, J. Biol. Chem. **99**: 717, 1927.

THE SEDIMENTATION RATE IN EXPERIMENTAL ANEMIA (RABBIT)*

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THE usefulness of the sedimentation test rests on the assumption that the speed of sedimentation is in direct proportion to the degree of tissue damage. Obviously the conditions under which the test is performed must be controlled, or an erroneous interpretation may be given the result of the procedure.

The effect of anemia alone upon the rate is not altogether clear. All workers are agreed that severe grades of anemia in the human subject are accompanied by accelerated rates. In less severe grades of anemia there is still some discussion as to whether the speed of sedimentation increases in direct proportion to the degree of anemia or not. Fåhraeus[1] and Cutler[2] have expressed the view that in the absence of other pathology there is some compensatory mechanism present which tends to keep the sedimentation rate relatively constant in moderate anemia. Gram,[3] Rourke and Ernstene,[4] Wintrobe,[5] and Walton,[6] on the basis of dilution experiments with cells in their own plasma, have been able to construct graphs showing the relation of various grades of anemia to sedimentation rate. It is important to realize that they were using suspensions of normal cells in normal plasma, not true anemic blood. We are presenting the results of experiments showing the behavior of the sedimentation rate in the anemia of acute and chronic hemorrhage and in recovery from that anemia.

It will be shown by successive readings on the *sedimentation of whole blood* that there is a marked increase in sedimentation rate in acute anemia, but only a slight increase in chronic anemia. Results of observations on the *sedimentation of suspensions of cells in plasma* will show the quantitative change in the factors affecting the sedimentation rate which are attendant upon the anemia produced. In general there will be noted a slowing of the sedimentation of suspensions as the anemia progresses and an acceleration in recovery.

Methods.—Six male rabbits were used, four New Zealand whites, weighing from 2,350 to 3,500 gm., and two Champagnes d'Argents, weighing 3,300 and 3,500 gm. The animals were housed in individual cages and fed a diet of oats and celery throughout the course of the experiments. On the first and second days of each experiment, the animal was bled from the ear vein approximately 15 to 20 c.c. of blood per kilo of body weight. Subsequently, he was bled approximately three times a week to maintain a fairly constant degree of anemia. Sedimentation rate, hemoglobin, hematocrit value, complete red and white cell counts, and differential counts were determined on the first and second days, usually three times a week for the first two weeks, twice the third week, and approximately once a week thereafter.

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Received for publication, July 30, 1936.

For all determinations except differential counts, a 4.5 c.c. sample of blood was mixed with 0.5 c.c. of an anticoagulant mixture containing 1.2 per cent ammonium oxalate and 0.8 per cent potassium oxalate. This mixture gives a resulting percentage of the two salts which does not change the size of the red cells.[7] Sedimentation on whole blood and suspensions of 5 million, 4 million, 3 million and 2 million cells in plasma was determined in Wintrobe[5] tubes, filled to the 50 mm. mark. Readings were made at the end of one hour, two hours, and three hours. Occasionally, it was difficult to obtain an accurate reading in the tube containing the two million suspension at the end of one and two hours, but usually the level was clear-cut at the end of three hours. Therefore, only the three-hour reading is here reported. The suspensions were made as follows: If the red cell count was over 5 million cells per c. mm. of plasma, 2.0 c.c. of the blood-anticoagulant mixture was retained, the remainder centrifuged. If the red cell count was less than 5 million cells per c. mm., one hematocrit tube was filled to the 50 mm. mark and the remainder centrifuged at low speed. Sufficient of the supernatant fluid was added to or removed from the retained specimen to produce a resulting suspension of 4.5 million cells per c. mm. of the plasma anticoagulant mixture. This is equivalent to a suspension of 5 million cells per c. mm. of plasma alone. Four-tenths cubic centimeter of this suspension was mixed with 0.1 c.c. of supernatant fluid to produce a suspension equivalent to a four million suspension of cells in plasma. Subsequent dilutions in similar manner produced suspensions equivalent to three and two million cells per c. mm. of plasma. This method of making suspensions is that used by Walton.[6] Red and white cell counts were made on the original blood anticoagulant mixture in certified pipettes and counting chambers, correction being made for the 9:1 dilution. Hemoglobin was determined on the same specimen in the Haden-Hausser[8] hemoglobinometer, Clinical model, 1935, the same correction being made for dilution. The Wintrobe tube, filled to the 50 mm. mark with blood-anticoagulant mixture, and previously used for the determination of sedimentation rate of whole blood, was centrifuged at 3000 r.p.m. for twenty minutes for the determination of red cell volume, using the same correction as in the other determinations.

Differential counts were made on direct smears stained with a modification of the Jenner stain devised by William A. Groat.[14] In each instance 200 to 300 cells were counted, enumerating the percentage of filamented amphophiles, nonfilamented amphophiles, lymphocytes, large mononuclears, eosinophiles, and basophiles. The criterion for differentiating filamented and nonfilamented cells is that used by Cook and Ponder and revised by Farley, St. Clair, and Reisinger.[9]

Total blood proteins were determined on three experiments. For this determination, 5.0 per cent sodium oxalate was used as anticoagulant, the protein estimated according to the colorimetric method described by Hawk and Bergeim.[10]

Three animals were autopsied after varying periods of anemia, three allowed to recover.

Results.—In Table I are shown the complete data on a typical animal subjected to repeated bleedings and allowed to recover (Exper. 36.5). Fig. 1 shows

in graphic form the relation of red cell count, volume, and hemoglobin to the sedimentation rate of whole blood and suspensions of cells in plasma in this animal.

In Table II are shown the complete data on a typical animal subjected to repeated bleedings and autopsied on the forty-third day. Fig. 2 is a graphic representation of the relation of the red cell count, volume, and hemoglobin to the sedimentation of whole blood and of suspensions of the cells in plasma for the first seventeen days of the experiment.

Tables IV to VII show the abbreviated data on the remaining four experiments with omission only of the white blood counts and differential counts.

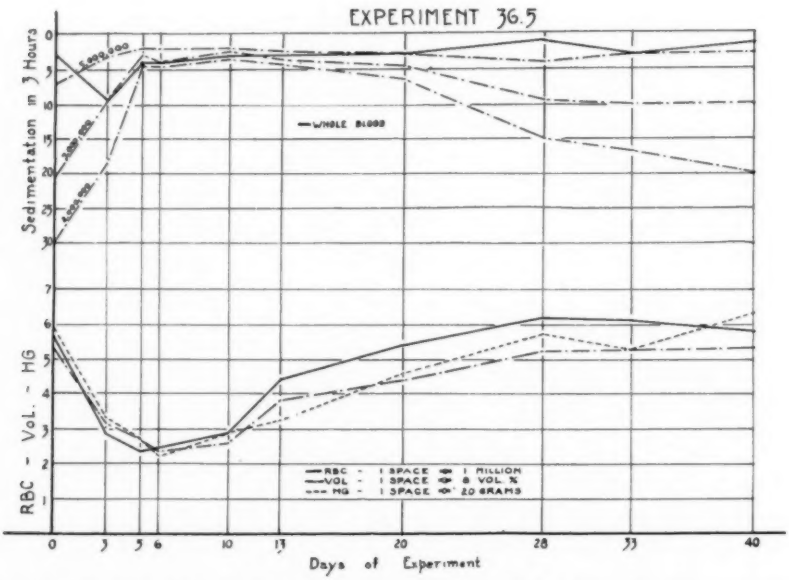
EXPERIMENT 36.5
Sedimentation in 3 Hours
WHOLE BLOOD
RBC - VOL. - HG
RBC - 1 SPACE = 1 MILLION
VOL - 1 SPACE = 8 VOL. %
HG - 1 SPACE = 20 GRAMS
Days of Experiment

Fig. 1.—The top graph shows the sedimentation of whole blood and of suspensions of 5 million, 3 million, and 2 million cells in plasma. The lower graph shows the red cell count volume per cent and hemoglobin at the various stages in the experiment.

Table III shows the relation of red cell count and sedimentation rate in all six animals for the first eleven days. Fig. 3 shows the relation of the average actual sedimentation observed in four animals (Expers. 35.19, 36.3, 36.4, and 36.5) with the expected rate on the basis of dilution of cells in plasma at the beginning of the experiments. In construction of the graph, Exper. 36.1 was disregarded because there were no readings between the sixth and eleventh days. Experiment 36.2 was omitted, since one ear was accidentally burned on the fifth day, and this was reflected in an increased rate. Throughout the remainder of their course these two animals gave sedimentation rates which closely paralleled the values observed in the other four animals.

In all six experiments there was noted an acceleration of the sedimentation rate of whole blood on the third day following the initial bleeding. This increase approximated the rate found in a suspension of cells of similar concentration at the beginning of the experiment. In the following readings there was seen a

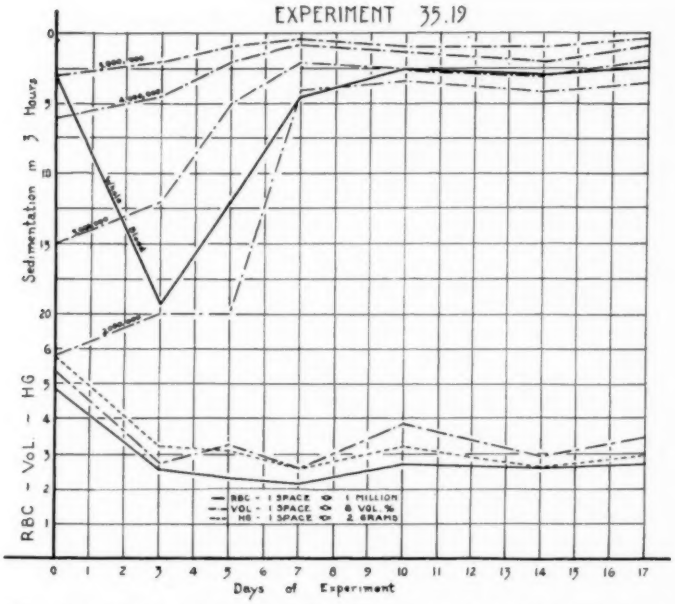
EXPERIMENT 35.19
Sedimentation in 3 Hours
RBC - VOL - HG
RBC - 1 SPACE ⇔ 1 MILLION
VOL - 1 SPACE ⇔ 8 VOL %
HG - 1 SPACE ⇔ 2 GRAMS
Days of Experiment

Fig. 2.—The top graph shows the sedimentation of whole blood and suspensions of 5 million, 4 million, 3 million, and 2 million cells in plasma. The lower lines show the red blood count, volume per cent, and hemoglobin. Note the gradual slowing of the sedimentation of the suspensions.

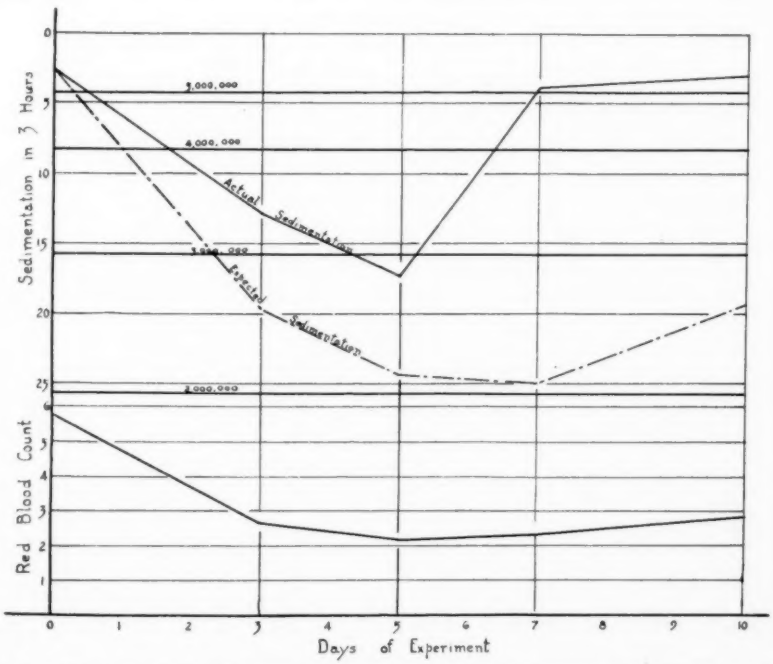

Fig. 3.—The top black line represents the average sedimentation of whole blood of the four experiments (Expers. 35.19, 36.3, 36.4 and 36.5). The shaded line shows the expected sedimentation *at that blood count* on the basis of the readings on suspensions at the beginning of the experiments. The average values of sedimentation of the various suspensions at the beginning of the experiments is shown by the transverse lines labeled 5,000,000, 4,000,000, 3,000,000, and 2,000,000. The lower graph records the red cell counts in millions throughout the first ten days of these experiments.

TABLE I

EXPERIMENT 36.5

Effect of anemia due to hemorrhage upon sedimentation rate.
Experiment begun on March 14 and ended April 23.
Male, white rabbit, weight 2,350 grams.

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	W.B.C. THOUSANDS	SEDIMENTATION IN MM. SUSPENSIONS					FIL. %	NON-FIL. %	LYMPH. %	MONO. %	EOS. %	BAS. %	PLASMA PROTEIN GR. PER 100 C.C.	WT.
						WHOLE BLOOD	MILLIONS											
							5	4	3	2								
0*	40.0	43.0	12.0	5,72	6,2	3.0	7.0	13.0	21.0	30.0	23.0	8.0	63.5	1.5	1.5	2.5		2350
1	25.0																	
3	10.0	25.0	6.4	2,93	5,3	9.0	3.0	5.0	9.0	18.0	19.3	2.3	72.7	1.3	1.3	3.0	4.9	
5	20.0	22.0	5.4	2,34	4,5	4.0	2.0	2.0	3.0	4.5	15.0	5.0	73.5	1.0	1.5	4.0		
6	15.0	19.0	4.4	2,44	5.5	4.0	2.0	3.0	4.0	4.5	13.0	3.0	76.5	1.5	1.0	5.0	4.7	
10	25.0	22.0	5.8	2,94	4,7	3.0	2.0	2.0	2.5	3.5	9.5	1.5	83.5	0.0	3.0	2.5	5.5	
13*	10.0	31.0	6.5	4,46	5,1	3.0†	2.5	3.0	3.5	4.0	9.0	2.0	81.5	2.0	1.5	4.0	6.1	
20	4.5	35.0	9.0	5,40	6,1	3.0	3.0	4.5	4.5	6.5	9.0	4.5	77.0	3.0	0.0	6.5		
28	4.5	42.0	11.4	6,18	5,7	1.5	4.0	5.5	9.0	15.0	13.5	5.0	70.5	5.5	1.0	5.5		2075
33	4.5	42.0	10.5	6,15	8,0	3.0	3.0	5.0	10.0	17.0								
40	4.5	44.0	12.8	5,87	6,2	2.0	3.0	6.0	10.0	20.0	22.5	7.5	41.0	5.5	1.0	22.5		2100

*Photographed.
†Sedimentation calculated from results of suspensions, not determined on whole blood.

TABLE II

EXPERIMENT 35.19

Effect of anemia due to hemorrhage upon the sedimentation rate.
Experiment begun on Feb. 12, ended March 26.
Male, white rabbit, weight 3,400 grams.

DAY	BLED C.C.	VOL. %	HC GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION IN MM. (3 HR.) SUSPENSIONS					W.B.C. THOUSANDS	FIL. %	NON-FIL. %	LYMPH. %	MONO. %	EOS. %	BAS. %	PLASMA PROTEIN GR. PER 100 C.C.	WT.
					WHOLE BLOOD	MILLIONS												
						5	4	3	2									
0	65.0	43.0	11.8	4,93	3.0	3.0	6.0	15.0	23.0	10,2	21.3	12.0	62.7	1.0	0.7	2.3	6.0	3400
1	55.0																	
3	55.0	22.0	6.4	2,60	19.0	2.0	4.5	12.0	20.0	10,3	20.3	7.3	64.4	1.0	0.7	5.3		3350
5	45.0	25.5	6.2	2,25	12.0	1.0	2.0	5.0	20.0	8,9	8.7	4.3	77.3	0.3	0.3	9.4		
7	43.0	21.0	5.2	2,10	4.0	0.5	1.0	2.0	3.5	5,5	17.7	8.0	67.0	1.3	0.7	5.3		
10	55.0	31.0	6.5	2,73	2.5*	1.0	1.5	2.5	3.0	4,6	10.0	5.3	78.3	0.0	1.0	5.5	7.1	3500
13	55.0																	
14	10.0	23.5	5.2	2,60	3.0	1.0	2.0	3.0	4.0	6,2	8.3	4.7	82.3	0.0	1.0	5.7	5.8	
15	40.0																	
17	54.0	27.0	5.9	2,70	2.5*	0.5	1.0	2.0	3.5	7,2	22.5	10.5	57.0	0.5	2.5	7.0	5.6	
19	26.0																	
21	43.0	24.0	5.8	3,32	3.0	1.0	1.5	3.0	3.0	3,3	17.3	4.0	72.0	0.3	2.7	2.0	6.2	
24	45.0																	
28	60.0	39.0	7.5	4,60	1.0	1.0	1.5	2.0	3.0	8,0	15.0	4.5	59.5	1.0	2.0	18.0	5.7	
31	45.0																	
33	35.0																	
35	60.0																	
38	10.0	29.0	6.2	4,20	2.0	1.5	2.0	3.0	3.5	5,9	14.0	3.0	79.0	2.0	1.0	1.0	4.9	
41	47.0																	
43	15.0	28.0	5.7	4,46	2.0	2.0	2.5	4.0	5.0	7,3	22.5	7.5	62.0	1.0	1.5	6.5		3525

*Sedimentation calculated from suspensions, not determined on whole blood.

TABLE III

THE RELATION OF THE RED BLOOD CELL COUNT TO SEDIMENTATION OF WHOLE BLOOD. ALL SIX EXPERIMENTS

DAY	EXPER. 35.19		EXPER. 36.1		EXPER. 36.2		EXPER. 36.3		EXPER. 36.4		EXPER. 36.5	
	R.B.C. MILLIONS	SED. MM.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.
0	4.93	3.0	5.34	3.0	4.80	4	5.80	2.5	6.50	2.0	5.72	3.5
3	2.60	19.0	3.36	8.0	2.70	14†	2.19	12.0	2.60	12.0	2.93	9.0
5	2.25	12.0			2.90	3†	1.90	36.0			2.34	4.0
6			3.50	3.5*							2.44	4.0
7	2.10	4.0					1.90	4.0	2.25	4.0		
8					2.76	6†						
9									3.40	3.0		
10	2.73	2.5					2.26	3.5			2.94	3.0
11			4.37	1.5					2.30	3.0		
12					3.10	3.5						
13			3.12	3.5								

*Readings estimated from results of suspensions, not actually determined on whole blood.
†Ear showed some burn.
In construction of the graph in Fig. 3 only values actually found on the 0, 3, 5, 7, and 10th days were used (Expers. 35.19, 36.3, 36.4, and 36.5).
R. B. C. recorded in millions.
Sedimentation recorded in millimeters.

TABLE IV

EXPERIMENT 36.1
Male White Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					WEIGHT
					WHOLE BLOOD	SUSPENSIONS MILLIONS				
						5	4	3	2	
0	30	39.0	11.6	5,34	-	3.0	5.0	8.0	12.0	2510
1	28									
3	5.0	25.0	7.0	3,36	-	3.0	5.0	10.0	13.0	
6	27.0	31.0	8.0	3,50	-	2.0	3.0	4.0	13.0	
11	39.0	36.0	9.0	4,37	1.5	1.5	2.5	3.0	5.0	
13	22	29.0	7.2	3,12	3.5	0.5	2.0	3.5	5.0	
17	54	34.0	9.0	4,09	2.5	1.5	2.5	3.0	4.0	
20	48	29.0	7.5	2,99	3.0	1.0	2.0	3.0	5.0	
34	30	28.0	6.8	3,30	-	1.5	2.0	3.0	4.0	
37	35	29.0	6.9	3,50	-	1.0	2.0	3.0	4.0	2460
41	30	33.0	8.0	3,42	2.0	1.5	2.0	3.0	5.5	
44	38	36.0	7.0	3,40	7.0	1.0	2.0	2.5	4.0	
46	28									
48	33	-	7.5							
51	15	26.5	7.0	3,50	2.0	2.0	2.5	4.0	5.0	
53	40									
55	38	29.0	7.5	3,84	2.5	1.5	2.0	3.0	4.5	
57	40									
59	29	-	5.4							
62	26	29.0	6.4	3,64	2.0	1.0	1.5	2.0	3.0	
64	33									
71	10	32.0	7.4	5,10	2.0	2.0	2.5	3.0	3.0	
76	10	42.0	10.0	5,09	1.0	1.0	1.5	2.5	2.5	
83	5	42.0	10.0	4,96	1.0	1.0	2.0	3.5	3.5	
90	5	48.0	12.0	6,64	1.5	1.5	2.5	3.5	5.0	
97	5	46.0	12.2	6,85	1.5	2.0	3.0	5.0	7.0	
104	10	49.0	13.8	7,92	1.5	3.5	4.5	10.0	12.0	
111	5	46.0	11.5	5,72	2.0	5.0	7.5	10.0	14.0	
119	5	41.0	11.6	8,92	2.5	-	6.0	10.0	15.0	2625

gradual slowing of the rate in both the whole blood and in the various suspensions. On the seventh day in four experiments the rate of sedimentation of the anemic blood, as drawn, closely approximated that found in normal whole blood at the beginning of the experiments. The omission of the reading between the sixth and eleventh days in Exper. 36.1 probably meant the missing of the first slowing of the rate. One ear was accidentally burned in Exper. 36.2, and this was reflected in a change in the rate.

In recovery from the anemia (Expers. 36.1, 36.4, and 36.5), there was a gradual acceleration of sedimentation of the various suspensions. The rates found in whole blood remained slow. The rate of suspensions in Exper. 36.1

TABLE V

EXPERIMENT 36.2
Male White Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					WEIGHT
					WHOLE BLOOD	SUSPENSIONS MILLIONS				
						5	4	3	2	
0	46	36.0	9.8	4,80	4.0	4.0	9.0	13.0	22.0	2560
1	41									
*3	39	24.0	5.4	2,70	14.0	2.0	4.0	8.0	-	
X5	40	24.5	5.8	2,90	3.0+	0.5	2.0	2.5	3.5	
H8	44	25.5	5.6	2,76	6.0	0.5	5.0	8.0	13.0	
12	38	28.8	5.8	3,10	3.5	0.5	1.5	3.0	6.0	
15	50	27.0	5.9	3,30	-	1.5	2.0	3.0	4.0	2300
19	31	29.0	7.2	4,24	2.0	1.0	2.0	3.5	4.0	
22	30	29.0	6.9	3,70	2.5	2.0	2.5	3.0	3.5	
26	23									
27	30									
29	40	24.5	6.4	3,40	3.0+	1.0	2.0	3.5	4.5	
33	25	-	4.8							
36	30	31.0	6.2	3,66	-	1.0	1.0	2.0	3.0	
41	35	27.0	6.1	4,20	2.5	2.0	2.5	3.5	4.5	
43	22									
46	28	-	5.4							2550
48	10	18.0	4.4	3,10	2.0	2.0	2.5	3.0	3.5	
Autopsy										

*Marked redness about base of left ear.
X, Demarcating crusted area approximately 1.0 cm. in diameter over the left ear.
H, Black slough 1.0 cm. in diameter at base of ear.

TABLE VI

EXPERIMENT 36.3
Male Gray Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					PLASMA PROTEIN	WT.
					WHOLE BLOOD	SUSPENSIONS MILLIONS					
						5	4	3	2		
0	65	49.0	13.8	5,80		3.0	5.0	11.0	23.0	7.6	3550
1	70				2.5						
3	62	20.5	5.3	2,19	12.0	2.0	3.0	5.0	12.0	7.1	
5	36	18.0	4.2	1,90	36.0	1.5	2.0	5.0	22.0	5.9	
7	10	16.0	4.4	1,90	4.0	1.0	2.0	4.0	6.0	7.2	
10	15	19.0	3.9	2,26	3.0+	-	2.0	4.0	5.0		
16	10	24.5	5.3	4,10	2.0	1.5	2.0	2.5	3.0		
21	10	30.0	5.8	4,25	2.0	1.5	2.0	3.0	3.0	4.9	
24	10										
27	10	24.0	5.4	4,36	-	2.5	2.5	3.0	4.0	5.0	2950

at the end of the experiment was slightly faster than at the beginning. In the other two experiments, the rates did not quite increase to the original level. As noted by other workers,[11, 12] following acute blood loss there was a slight increase in both color and volume indices. In the period of chronic hemorrhage, the color indices fell more than the volume indices, giving saturation indices below 0.8, which has been shown to be characteristic of anemia of chronic blood loss.[13] In recovery the red cell count rose in each instance above the original level. In the animals kept anemic longer, this polycythemia was greater. The color indices rose gradually throughout the period of recovery to approximate the original values.

Tables IV, V, VI, and VII show the relation of red cell count, hemoglobin, and volume to sedimentation of whole blood and suspensions in the remaining experiments. The white blood picture showed no constant or significant change. The total count per cubic millimeter varied between 3,400 and 11,000, both extremes of which may be considered within the range of normal. Following the initial bleedings in two animals, there was a slight increase in the percentage of amphophiles, and in one animal an increase in nonfilamented amphophiles to over 50 per cent.

In three experiments plasma proteins were determined. These showed little or no change for the first seven days. Later there was an irregular decrease in the period of sustained blood loss.

Autopsy on three animals (Expers. 36.2, 36.3, and 35.19), after forty-eight days, twenty-seven days, and forty-three days, respectively, showed only generalized pallor of all the organs on gross examination. The marrow of the femora appeared very red and gelatinous. The tibial marrow was rather markedly reddened throughout the upper two-thirds, the remainder appearing rather fatty.

TABLE VII

EXPERIMENT 36.4
Male White Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					PLASMA PROTEIN	WT.
					WHOLE BLOOD	SUSPENSIONS MILLIONS					
						5	4	3	2		
0	52.0	49.0	13.0	6,50	2.0	4.0	9.0	16.0	27.0	9.0	3700
1	33.0										
3	32.0	21.0	5.5	2,60	12.0	3.0	5.0	11.0	32.0	6.5	
5	25.0	21.0	5.5	3,30	–	–	–	–	–	8.0	
7	5.0	17.0	4.1	2,25	4.0	2.0	3.0	4.0	7.0	7.4	
9	10.0	16.0	3.9	3,40	4.0	3.0	3.0	3.5	5.0	4.9	
11	10.0	19.0	4.1	2,30	–	2.0	–	–	3.0	–	
13	10.0	22.0	3.75	2,67	3.0	1.0	2.0	2.5	3.0	4.8	
18	30.0	–	5.2	–	–	–	–	–	–	–	
20	10.0	20.5	3.9	3,40	2.5	1.5	2.0	2.5	3.0	6.1	
29	4.5	24.5	5.8	5,10	5.0	5.0	6.0	7.0	8.0	4.4	
36	4.5	27.0	6.5	5,70	5.0	4.0	6.0	8.0	13.0		
43	4.5	34.0	7.0	7,90	2.0	–	6.0	10.0	11.0		
50	4.5	35.0	8.0	6,63	2.0	4.0	8.0	12.0	18.0		3425
57	4.5	41.0	9.0	6,93	1.5	3.0	2.0	6.5	16.0		
71	9.0	39.5	12.0	7,90	1.0	2.5	3.5	7.0	6.0		
78	4.5	49.0	12.4	7,10	2.0	4.5	8.0	12.0	16.0		

Microscopic examination showed marked diffuse hyperplasia of all marrow sections (femora). The erythroblastic series appeared to predominate.

Although the number of animals used in this work is small, the uniformity of results appears to justify this report.

SUMMARY

The following changes in the sedimentation rate were observed in anemia produced by hemorrhage in rabbits:

1. In *acute anemia* an *acceleration* of the sedimentation rate of *whole blood* in nearly direct proportion to the degree of anemia;

2. In *chronic anemia* a gradual *slowing* of the sedimentation rate of both *whole* (anemic) *blood* and of *suspensions* of cells in plasma, which reached a relatively constant slow rate on and after the seventh day;

3. In *recovery* from chronic anemia a gradual *acceleration* in the sedimentation rate of *suspensions* of cells in plasma, which lagged behind the rise in red cell count.

Changes in the red cells were similar to those noted by other observers.

No significant changes were found in the total white blood count, differential count or in the filament-nonfilament ratio.

REFERENCES

1. Fåhraeus, Robin: The Suspension Stability of the Blood, Physiol. Rev. **9**: 241, 1929.
2. Cutler, J.: Personal communication, 1935.
3. Gram, H. C.: Sedimentation of Blood Corpuscles in Various Internal Diseases and Result of Correction of This Value for Variations of Hemoglobin Percentage, Acta. med. Scandinav. **70**: 242, 1929. Abstr. in J. A. M. A. **92**: 2070, 1929.
4. Rourke, M. D., and Ernstene, A. C.: A Method for Correcting the Erythrocyte Sedimentation Rate for Variations in the Cell Volume Percentage of Blood, J. Clin. Invest. **8**: 545, 1930.
5. Wintrobe, M. M., and Landsberg, W.: A Standardized Technique for the Blood Sedimentation Test, Am. J. M. Sc. **189**: 102, 1935.
6. Walton, A. C. R.: The Corrected Erythrocyte Sedimentation Test, J. Lab. & Clin. Med. **18**: 711, 1933.
7. Heller, V. G., and Paul, H.: Changes in Cell Volume Produced by Varying Concentrations of Different Anticoagulants, J. Lab. & Clin. Med. **19**: 777, 1934.
8. Haden, Russell: New Clinical Model of the Haden-Hausser Hemoglobinometer, J. Lab. & Clin. Med. **20**: 762, 1935.
9. Farley, D. R., St. Clair, H., and Reisinger, J. H.: The Normal Filament and Non-filament Polymorphonuclear Neutrophil Count; Its Practical Value as a Diagnostic Aid, Am. J. M. Sc. **180**: 336, 1930.
10. Hawk, Philip B., and Bergeim, Olaf: Practical Physiological Chemistry, ed. 10, Philadelphia, P. Blakiston's Son and Co., Inc., pp. 449, 450.
11. Price-Jones, Cecil: Observations on the Changes Produced in the Blood and Bone Marrow by Hemorrhage and Phenyl Hydrazine. Part 1. J. Path. & Bact. **15**: 4, 1911.
12. Whipple, G. H., Hooper, C. W., and Robscheit, F. S.: Blood Regeneration Following Simple Anemia. 1. Mixed Diet Ration, Am. J. Physiol. **53**: 151, 1920.
13. Osgood, E. E., and Haskins, H. D.: Causes, Classification and Differential Diagnosis of Anemias. Based on the Detailed Examination of Over Two Hundred Patients and a Study of the Literature, Ann. Int. Med. **5**: 1367, 1932.
14. Groat, W. A.: A General Purpose Polychrome Blood Stain, J. Lab. & Clin. Med. **21**: 978, 1936.

SOME SYMPATHETIC FACTORS IN THERMOTHERAPY*

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INTRODUCTION

THE production of infectious diseases in mental patients was first attempted by Rosenblum in 1875. Patients who were suffering from different mental disorders were inoculated with relapsing fever organisms. Later, Von Jauregg studying the results of epidemics occurring in mental hospitals, noted that when these patients contracted infectious diseases accompanied by a high fever, the tendency in nearly every case was to recover from their psychosis.

Stimulated by this discovery, Von Jauregg, in 1887, first proposed the treatment of paresis by the malarial method. In the beginning he realized the severity of this method of treatment and resorted to various toxins to produce artificial fever. The results obtained from the use of various toxins were not entirely satisfactory and he returned to his original idea of malarial inoculations.

Torres in 1913 reported successful results in the treatment of typhoid fever by giving typhoid vaccine intravenously and described the sharp reactions that followed its injection. Typhoid vaccine had been used previous to this in the treatment of typhoid, but the injections had always been given subcutaneously.

Dessy, Grapiolo, and Fossati in 1914 published a paper on the treatment of typhoid fever with typhoid vaccine. The authors compared the results obtained by the subcutaneous and intravenous injections of typhoid vaccine. They concluded that the intravenous method gave much quicker results.

Kraus and Mazza in 1914 treated typhoid fever with intravenous injections of typhoid vaccine and noted that the mortality rate was definitely reduced. Ichikawa also reported favorably on this form of treatment. At this time many believed that this method of vaccine treatment was originally intended as a form of specific therapy; but when Kraus and his coworkers in 1917 obtained similar results with colon bacillus vaccine, they opened the door to nonspecific protein therapy in all of its various forms and modifications.

Miller and Lusk in 1916 were the first to use protein as a nonspecific therapy in the United States. They reported favorably on the intravenous use of typhoid vaccine in arthritis.

Vaughan in 1916 maintained that the fever produced by protein therapy was due to the parenteral destruction of the induced protein. He pointed out that the parenteral introduction of bacteria in any form was followed by fever and that fever was produced by the splitting up of the bacterial protein.

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Received for publication, July 31, 1936.

FEVER THERAPY METHODS

The first report of fever therapy being used in the treatment of mental disorders in the United States appeared in 1922 at Saint Elizabeth's Hospital, Washington, D. C. Since that time, a large number of mental institutions in this country have inaugurated fever therapy as a treatment for various psychoses.

A number of theses have been written on the various methods of producing artificial fever with malaria, ratbite fever organisms, typhoid vaccine, Coley's solution, sulphosin, diathermy, radiotherapy, radiant energy, and hydrotherapy. All these methods have reported various degrees of success. Each method seems to bring out a new theory as to the reaction that causes the fever and the effect it has on the patient.

To observe some of these factors produced by artificial fevers, we have used for the past two years typhoid-paratyphoid vaccine prepared by Parke, Davis Company. The vaccine contains 2,000 million killed bacteria per cubic centimeter prepared specially for intravenous injections. The vaccine is easily diluted, readily administered, and has no drastic effects. The injections are given intravenously once a week. The initial dose in most cases is from 10 to 20 million bacteria, depending upon the weight of the patient. The stock vaccine is diluted with sterile triple distilled water and the desired amount given with a tuberculin syringe.

We have found that the most desirable results are obtained by adhering to the following schedule of injections:

10 million bacteria the first week
20 million bacteria the second week
40 million bacteria the third week
80 million bacteria the fourth week
100 million bacteria the fifth week
Increasing the dose a hundred million bacteria each week until eighteen doses have been given.

This schedule should be applied only in psychotic cases where there is no evidence of an active somatic disease. Cecil (1935) gives a complete list of important contraindications which should be considered previous to the intravenous use of protein therapy.

THE PRESENT STUDY

During the past two years one hundred selected patients with various psychoses have received typhoid-paratyphoid vaccine therapy. What is meant by selected patients is that only those individuals who were free from any somatic diseases received the therapy. In our group there were 37 males and 63 females. The average age was thirty-six years. The oldest patient was sixty-eight and the youngest was sixteen. The average time that the entire group had been in their psychosis when the therapy started was five years and six months.

The literature on the regulation of the blood elements by the vegetative nervous system was excellently summarized by Ferdinand Hoff in 1928.

THE EFFECT OF FEVER THERAPY ON THE LEUCOCYTES

After the patients responded to the fever therapy, six hundred white blood cell counts, differential counts, and Arneth counts were done at various time intervals. Standard methods were used for the white blood cell counts and differential counts. The figures under the Arneth count represent the average percentage of lobes present in the polymorphonuclears. The figure 1.86 would indicate a shift to the right, denoting a mature neutrophile, while 1.38 would indicate a shift to the left which is indicative of the proliferation of juvenile neutrophiles. Our normal was established on twenty individuals with normal white and red blood cell concentrations to be 1.70.

TABLE I

THE AVERAGE VALUES OF SIX HUNDRED WHITE BLOOD CELL COUNTS, DIFFERENTIAL COUNTS AND ARNETH COUNTS TAKEN AT VARIOUS TIME INTERVALS

	W.B.C.	PER CENT OF NEUTROPHILES	ARNETH COUNT
One hour before therapy	8,500	58.6	1.86
One hour after therapy	6,931	53.1	1.88
Two hours after therapy	11,891	83.0	1.59
Three hours after therapy	19,684	93.7	1.38
Six hours after therapy	11,042	75.3	1.57
Twenty-four hours after therapy	9,300	64.0	1.66

THE EFFECT OF FEVER THERAPY ON THE RETICULOCYTES

Schleicher in 1933 reported a new method of counting reticulocytes which seems, for all clinical purposes, to be quite satisfactory. Schleicher found, after a careful study of several hundred smears made from normal human bloods, showing balanced red blood cell and hemoglobin concentrations, the normal range of reticulocyte concentration to be from 2 to 3 per cent. The limits of error in the method employed were plus or minus 1 per cent. In his article he gives a detailed account of the stains and method of enumeration. We utilized Schleicher's method for the determination of the number of reticulocytes present in the patient's blood at the various time intervals while undergoing fever therapy.

TABLE II

THE AVERAGE VALUES OF ONE HUNDRED AND FIFTY RETICULOCYTE COUNTS TAKEN AT VARIOUS TIME INTERVALS

One hour before therapy	2.37 per cent
One hour after therapy	1.76 per cent
Two hours after therapy	2.57 per cent
Three hours after therapy	6.35 per cent
Six hours after therapy	5.34 per cent
Twenty-four hours after therapy	2.72 per cent

THE EFFECT OF FEVER THERAPY ON THE BLOOD SUGAR LEVELS

A survey of the literature bearing on the problem of the blood sugar level of individuals receiving thermotherapy is rather limited. However, volumes have been written on the various phases of blood sugar in both health and disease. The phase that we are most interested in is the blood sugar levels in mental conditions including the emotional disorders.

In a series of 148 cases of mental diseases of various kinds with different types of emotional states studied by Bowman and Kasanin (1929), the majority showed normal blood sugar values (80 to 120 mg. dextrose per 100 c.c. of whole blood) in contrast to results of former investigators. The distribution curve was normal.

Blanche Labin (1927) reported on glycemia in the insane and stated that the variations from the normal limits were only slight.

Craig (1927) reported that no characteristic blood sugar curve was found in 90 patients with anxiety neuroses, epilepsy, and melancholia.

In 100 cases of manic depressive psychosis studied by Rothschild and Malamud (1931) and uncomplicated by somatic diseases, there was no constant relation between the ratios of distribution of sugar, bromine, calcium, and chlorides.

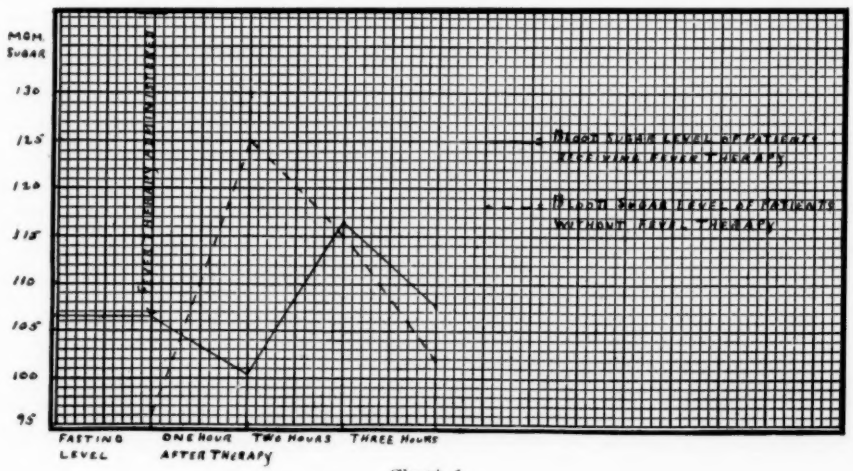

Chart 1.

An extensive study presented by Freeman (1933) of the fasting blood sugar levels as disclosed by six samples taken at standard intervals from 59 male schizophrenic patients over a period of six and one-half months, showed that 95 per cent of 347 determinations lay between the conventional limits of normality.

In dementia precox patients treated with sulphur, Isikowitz (1934) points out the behavior of the blood sugar by showing that six hours after the injection of sulphosin there is a lowering of the sugar. The hyperthermic phase following the injection is frequently accompanied by hyperglycemia.

In our group of 100 cases of various psychoses, 30 patients were observed for changes in the blood sugar. For the determination of the blood sugar level at the various time intervals, we used the approved method of Folin-Wu. The first blood was taken before breakfast (fasting level), and immediately after the blood sample was removed, the patients received 6 ounces of whole milk, 2 soda crackers, and an intravenous injection of typhoid-paratyphoid vaccine. Another blood sample was withdrawn at one, two, and three hours after the fever therapy.

In order to determine the limits of the blood sugar concentration in patients not undergoing fever therapy, a group of 10 patients were used as controls. This group were given the same breakfast but no fever therapy.

Chart 1 shows the average values of 120 blood sugar determinations at the various time intervals on 30 patients undergoing fever therapy. Superimposed thereon are the average values of 40 blood sugar determinations at various time intervals on 10 patients without fever therapy.

THE EFFECT OF FEVER THERAPY ON THE BLOOD PRESSURE, PULSE AND RESPIRATION RATES

Whitehorn (1934) states that in recent studies on the heart in emotional reactions, he has encountered some evidence that the cardiac accelerations associated with brief emotional reactions may be negotiated through central inhibition of the vagus, rather than through sympathetic activity. From the standpoint of its value as a laboratory indicator, the heart is a sensitive indicator of the emotions, even when they are slight and brief, whereas the blood sugar has no practical value as an indicator, even in the most extreme emotional excitement; but this seems to be contrary to other findings.

TABLE III

THE AVERAGE VALUES OF 182 SYSTOLIC BLOOD PRESSURES TAKEN AT VARIOUS TIME INTERVALS ON PATIENTS WHO WERE REACTING TO FEVER THERAPY

Before fever therapy	115 mm. Hg
20 minutes after	103 mm. Hg
40 minutes after	100 mm. Hg
60 minutes after	102 mm. Hg
80 minutes after	109 mm. Hg
100 minutes after	110 mm. Hg
120 minutes after	104 mm. Hg
140 minutes after	106 mm. Hg
160 minutes after	104 mm. Hg
180 minutes after	103 mm. Hg
200 minutes after	102 mm. Hg
220 minutes after	107 mm. Hg
240 minutes after	110 mm. Hg

With Whitehorn's statement in mind, we made a careful investigation of the temperature, pulse, and respiration of patients while they reacted to fever therapy. The temperature, pulse, and respirations were recorded by registered nurses.

TABLE IV

THE AVERAGE VALUES OF 230 TEMPERATURES, PULSES AND RESPIRATIONS TAKEN AT VARIOUS TIME INTERVALS DURING FEVER THERAPY

	TEMPERATURE	PULSE	RESPIRATION
One hour before therapy	98.0° F.	80.5	16.8
One hour after therapy	96.9° F.	79.0	16.7
Two hours after therapy	100.2° F.	98.5	23.8
Three hours after therapy	100.7° F.	102.9	26.6
Six hours after therapy	98.6° F.	87.5	19.9
Twenty-four hours after therapy	98.1° F.	80.0	17.3

When a patient is responding to fever therapy, there is a hyperventilation of the lungs in an attempt to cool the body. This has the effect of removing

carbonic acid from the blood in the expiration of abnormal amounts of carbon dioxide and creates the state designed as uncompensated deficit of carbon dioxide. This fact was demonstrated with hot baths by Haldane and Priestley (1905), later confirmed by Hill and Flack (1909), and a number of other workers. Hopkins (1934) reported the average fall of the carbon dioxide combining power to be 9.37 per cent by volume during hyperpyrexial baths.

The marked loss of carbon dioxide from the blood is of such a degree as to give rise to a condition of alkalosis, which may be detected without difficulty in the rising pH values. This alkalosis during hyperpyrexia was reported by Cajori, Crouter, and Pemberton (1923).

Barbour (1921) subjected dogs to hot baths and observed a dilution of the blood to be about 10 per cent. About this fact he was led to build his conception of water-shifting between the tissues and the blood as a protection of the body against overheating. By this means larger quantities of blood were made available for filling the greatly dilated peripheral vascular bed, bringing more blood to the surface of the body for direct conduction and radiation of heat, as well as mobilizing water for loss of heat through evaporation of the sweat.

The period of well-being which patients experience following typhoid-paratyphoid therapy may well be accounted for by the stimulation of hematopoiesis and the shifting of water between the vascular system and the tissues.

THE EFFECTS OF THE DIVIDED DOSE METHOD OF FEVER THERAPY

Kirby (1926) reported that the beneficial effect of fever therapy in neurosyphilis was roughly proportional to the height of the temperature obtained. He believed that a temperature of about 105° F. was necessary in order to obtain the maximum results. Winslow, Miller and Noble (1916) believed that a high temperature, artificially produced, 104° F. or higher, has a favorable influence on an established infection, while lower temperatures would seem to retard the formation of immune bodies. In order to produce, artificially, temperatures of 104° F. or higher, Nelson (1931) used a technic which consisted in giving two daily intravenous injections of typhoid-paratyphoid vaccine. He suggested that "the first dose be given at any selected time and be of a size calculated to cause slight fever. The second is given during the height of the fever produced by the first . . . usually at the end of the second or third hour. The second dose seems to have the effect of exploding the charge supplied by the first and in this way relatively small doses are capable of producing fever apparently as high as desired . . . 105° F. to 107° F."

This method of daily divided doses of typhoid-paratyphoid vaccine was used by Driver and Shaw (1933) with very satisfactory results in cases of neurosyphilis.

Eight of our patients received once a week a divided dose as suggested by Nelson. The highest temperature obtained by this method was 104.8° F. The average temperature of the group was 104.3° F.

The blood counts, differential and Arneth counts on the patients who were treated by the divided dose method showed that the maximum stimulation of the hemopoietic system is not in direct proportion to the height of the temperature. Therefore, the single dose method outlined above is recommended.

TABLE V

THE AVERAGE VALUES OF THE TEMPERATURES, PULSES AND RESPIRATIONS TAKEN DURING THE FEVER CRISIS OF THE DIVIDED DOSE METHOD

	TEMPERATURE	PULSE	RESPIRATION
One hour before therapy	98.3° F.	73.2	16.6
First fever crisis	101.3° F.	96.2	23.2
Second fever crisis	104.3° F.	114.5	29.0

TABLE VI

THE AVERAGE VALUES OF THE WHITE BLOOD CELL COUNTS, DIFFERENTIAL COUNTS AND ARNETH COUNTS TAKEN DURING THE FEVER CRISIS OF THE DIVIDED DOSE METHOD

	W.B.C.	PER CENT OF NEUTROPHILES	ARNETH COUNT
One hour before therapy	7,950	55.6	2.06
First fever crisis	18,287	94.6	1.40
Second fever crisis	18,500	95.6	1.37

COMMENTS

At the present time, all methods of fever therapy are being studied from every available angle. Some investigators believe that the hyperpyrexia is the sole factor in bringing about good results. Others raise the question that if the hyperpyrexia is the sole factor, how can they account for the fact that more uniform results are not obtained. Among our group there were seven patients who did not respond to the typhoid-paratyphoid vaccine. They manifested no clinical symptoms and no changes were noted in their blood laboratory work.

We have found, using the single dose method, that within the first hour after the typhoid-paratyphoid vaccine has been administered the patient experiences a state of shock, accompanied by nausea and chills; also some cases become quite cyanosed. During the period of shock a marked leucopenia, a slight shift to the left in the Arneth count, no change in the reticulocyte count, a hypoglycemia and a decrease in the systolic blood pressure occur.

Shortly after the state of shock (from two to three hours after the therapy), the patient develops a moderately high fever. During this hyperpyrexia the leucopenia is replaced by a leucocytosis. At this time a decided shift to the left in the Arneth count, a marked increase in the reticulocytes, an increase in the blood sugar level and an increase in the systolic blood pressure occur.

Ferdinand Hoff (1931) has laid down the law that vagotonic or, more properly, parasympatheticotonic conditions incline to leucopenia, a lymphatic tendency and eosinophilia. Sympatheticotonic conditions on the other hand incline to leucocytosis, a myeloid tendency with a left shift of the nuclear count and a lowering of the eosinophiles.

If this law may be taken as a guide we may deduce the conclusion from our results that bacterial protein fever therapy has resulted in a primary parasympathetic stimulation, followed by a more marked and prolonged sympathetic stimulation.

The authors wish to express their gratitude to Thomas Verner Moore, for his cooperation and helpful suggestions in completing this study.

REFERENCES

Barbour, H. G.: Proc. Soc. Exper. Biol. & Med. **18**: 186, 1921.
Bowman, K. M., and Kasanin, J.: Arch. Neurol. & Psychiat. **21**: 2, 1929.
Cajori, F. A., Crouter, C. Y., and Pemberton, R.: J. Biol. Chem. **57**: 217, 1923.
Cecil, R. L.: J. A. M. A. **105**: 1852, 1935.
Craig, R. N.: Lancet **1**: 925, 1927.
Dessy, S., Grapiolo, F. L., and Fossati, V.: Notas exper. y clin. Semana med. **21**: 357, 1914.
Driver, J. R., and Shaw, H. C.: J. A. M. A. **101**: 26, 1933.
Haldane, J. B. S., and Priestley, J. G.: J. Physiol. **32**: 252, 1905.
Hill, L., and Flack, M.: J. Physiol. **38**: 1909. Proc. the Physiol. Soc. **3**: 27, 1909.
Hoff, Ferdinand: Blut und Vegetative Regulation, Ergebn. d. inn. Med. u. Kinderh. **33**: 195, 1928. Vegetatives Nervensystems und Blut in Lebensnerven und Lebenstriebe. Ed. by Müller, Berlin, 1931.
Hopkins, H.: Arch. Neurol. & Psychiat. **31**: 3, 1934.
Ichikawa, S.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **23**: 32, 1914.
Isikowitz, S.: Acta med. Scandinav. **82**: 2, 1934.
Kirby, G. H.: State Hosp. Quart. **11**: 1926.
Kraus, R., and Mazza, S.: Deutsche med. Wchnschr. **40**: 1556, 1914.
Kraus, R., Penna, J., and Bonorino, C. J.: Wien. klin. Wchnschr. **30**: 868, 1917.
Labin, B.: Compt. rend. Soc. de biol. **96**: 1, 1927.
Miller, J. L., and Lusk, F. B.: J. A. M. A. **66**: 1756, 1916.
Nelson, M. O.: Am. J. Syph. **15**: 185, 1931.
Rothschild, D., and Malamud, W.: Arch. Neurol. & Psychiat. **26**: 4, 1931.
Schleicher, E. M.: Am. J. Clin. Path. **3**: 375, 1933.
Torres, E. R.: Vacunoterapia o antigenoterpia antifica, Semana med. **22**: 1557, 1913.
Vaughan, V. C.: J. Lab. & Clin. Med. **2**: 15, 1916.
Von Jauregg, Ritter: Deutsche Ztschr. f. Norvenhilk. 11, 1926.
Winslow, C. A., Miller, J. A., and Noble, W. C.: Proc. Soc. Exper. Biol. & Med. **13**: 93, 1916.
Whitehorn, J. C.: Year Book of Neurology, Psychiatry and Endocrinology, 1934.

CHLORINE ION DETERMINATIONS ON VENTRICULAR FLUIDS, SUPPLEMENTED WITH A FEW CISTERNAL AND SPINAL FLUIDS IN COMPARISON WITH THE CORRESPONDING BLOOD SERUM*

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THE chlorine ion concentration of spinal fluid obtained by lumbar puncture has been the subject of numerous studies, while other fractions of the cerebrospinal fluid have been examined only a few times.

Cestan, Riser and Laborde have analyzed one single specimen of ventricular fluid; otherwise such analyses have been reported only by Fremont-Smith, Mary Dailey and others, who in their large material of spinal fluid examinations have 7 cisternal fluids and 6 ventricular fluids which showed the same chlorine ion concentration as was found in caudal portions of the cerebrospinal fluid.

The findings here presented form a part of a series of examinations aiming at a comparison of the concentration of various substances in the ventricular fluid and the corresponding blood serum, supplemented with a few specimens of spinal fluid withdrawn for encephalography and with two cisternal fluids. The blood is taken from a median vein of the arm, under paraffin, in the usual manner.

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Received for publication, April 29, 1936.

The analyses are carried out after the method given by Rehberg (a modification of Van Slyke's method). The principle of this technic is: 0.1 c.c. of serum or spinal fluid is mixed with 0.5 c.c. of 0.03 N solution of $AgNO_3$ and boiled in a water-bath for half an hour, with addition of perhydrol. After cooling, 1 as an indicator. The Rehberg microburette is employed in the titration. The c.c. of ether is added, and titration is performed with 0.1 N ammonium thiocyanate, with air being led through the tube and with ferric ammonium sulphate as an indicator. The Rehberg microburet is employed in the titration. The calculations are performed according to known solutions of NaCl.

Triple analyses are made, and the mean value is calculated therefrom. Only in exceptional instances is the experimental error of the individual analyses greater than 4 mg. per cent.

The tables give the preliminary results. The third and fourth columns give the results of the cell counts in 3 c.mm. which have always been made within one hour after withdrawal of the fluids.

The fifth and sixth columns give the chlorine ion concentration in milligram per cent. The last column gives the quotient (spinal fluid chlorine divided by serum chlorine).

Analyses of 30 ventricular fluids, supplemented with 8 spinal fluids and 2 cisternal fluids, show that the chlorine ion concentration is the same throughout

TABLE I

CHLORINE ION CONCENTRATION IN VENTRICULAR FLUIDS AND SERUM

CASE	DIAGNOSIS	R.B.C.	W.B.C.	VENTR. FL.	SERUM	QUOT.
1	Glioma of hemisph.	400/3	6/3	413.2	355.0	116
2	Suspicion of intracran. tumor	100/3	4/3	448.2	356.6	126
3	Spongioblastoma of hemisph.	400/3	6/3	445.6	374.5	119
4	Suprasellar dermoid	210/3	60/3	441.6	375.9	117
5	Suspicion of intracran. tumor	140/3	4/3	436.3		
6	Tuberous sclerosis	4500/3	35/3	437.9	358.1	122
7	Ependymitis	30/3	2/3	443.8	366.5	121
8	Suspicion of intracran. tumor	202/3	10/3	439.5	370.7	119
9	Suspicion of intracran. tumor	80/3	11/3	439.5	368.7	119
10	Ependymitis	290/3	4/3	447.3	372.8	120
11	Suspicion of intracran. tumor	1180/3	12/3	438.6	364.6	120
12	Suspicion of intracran. tumor	394/3	18/3	436.1		
13	Parasagittal meningioma	900/3	5/3	438.6	364.6	120
14	Glioma of the brain stem	680/3	25/3	441.1	366.7	120
15	Glioma of hemisph.	25/3	75/3	435.4	361.1	121
16	Glioma of hemisph.	98/3	6/3	450.1	356.8	126
17	Suspicion of intracran. tumor	140/3	16/3	457.6		
18	Venous aneurysm	12/3	6/3	462.4	373.5	124
19	Glioma of hemisph.	18/3	4/3	436.0		
20	Meningioma of petrous bone	340/3	5/3	468.1	388.7	120
21	Suspicion of intracran. tumor	356/3	4/3	456.8	372.8	123
22	Suspicion of intracran. tumor	400/3	210/3	455.0	381.4	119
23	Suspicion of intracran. tumor	216/3	3/3	451.6	377.1	120
24	Traumatic lesion of hemisph.	1680/3	5/3	448.2	366.5	122
25	Meningioma of convexity	550/3	12/3	449.5	368.2	122
26	Glioma of hemisph.	1150/3	18/3	457.5	370.5	123
27	Glioma of hemisph.	2500/3	6/3	448.2	365.9	122
28	Suspicion of intracran. tumor	620/3	2/3	451.0	374.4	120
29	Suspicion of intracran. tumor	60/3	1/3	452.2	373.6	121
31	Glioma of hemisph.	950/3	26/3	453.7	370.7	122

TABLE II

CHLORINE ION CONCENTRATION IN CISTERNAL AND SPINAL FLUIDS AND SERUM

CASE	DIAGNOSIS	R.B.C.	W.B.C.	FLUIDS	SERUM	QUOT.
C1*	Supracellar dermoid	50/3	24/3	429.7	363.2	118
C2*	Traumatic atrophy of the brain	6/3	13/3	434.8	357.0	122
L1	Traumatic atrophy of the brain	14/3	2/3	439.4		
L2	Suspicion of intracran. tumor	20/3	8/3	434.5		
L3	Suspicion of intracran. tumor	9/3	1/3	430.9	355.6	121
L4	Suspicion of intracran. tumor	7/3	3/3	438.0		
L5	Suspicion of intracran. tumor	800/3	3/3	446.1	370.4	120
L6	Suspicion of intracran. tumor	24/3	80/3	442.1		
L7	Glioma of hemisph.	75/3	6/3	442.0	352.8	125
L8	Suspicion of intracran. tumor	60/3	5/3	445.7	369.2	121

*Cisternal fluids.

the cerebrospinal fluid, being about 20 per cent higher than the corresponding value for serum.

The material for the analyses here reported was placed at my disposal from the Department of Neurology and Neurosurgery of the Rigshospital. I wish to give my thanks to Professor Viggo Christiansen and Dr. E. Busch for this material.

The analyses were carried out in the laboratory of the Department of Psychiatry, Rigshospitalet. I am greatly indebted to Professor Wimmer for his kind permission to work in the laboratory.

REFERENCES

Fremont-Smith; Mary Dailey, and others: Arch. Neurol. & Psychiat. **25**: 1271, 1931.
Rehberg, P. Brandt: Biochem. J. **20**: 483, 1926.

AN OUTBREAK OF FOOD POISONING PROBABLY DUE TO STAPHYLOCOCCUS AUREUS*

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ON Nov. 17, 1935, two score people were involved in a food poisoning outbreak in this city following the eating of cream puffs with custard filling. It was found that only the people who ate the cream puffs became ill. Other cream puffs from the same lot and coming from the same source were submitted to our laboratory for bacteriologic examination, the day after the food poisoning occurred. A direct microscopic examination showed the presence of a coccus and a large gram-positive spore-forming bacillus. Some of the filling from four cream puffs was planted, respectively, in four broth tubes. These were incubated at 37° C. for twenty-four hours and a loopful of each was spread over blood serum plates. Two types of colonies were fished to agar slants and after growth were examined morphologically. The yellow colonies proved to be staphylococci and the colorless colonies spore-bearing bacilli. The staphylococcus was run through the sugars ordinarily used for its differentiation. It gave the

*From the Bureau of Laboratories, Department of Health.
Received for publication, September 15, 1936.

expected reactions in all but one sugar—it did not ferment lactose. It liquefied gelatin and produced hemolysis on blood agar plates.

Aside from ruling out the anthrax bacillus, nothing further was done with the spore-forming bacillus, as we know of no instance in which organisms of the aerobic spore-forming group of bacilli (*B. subtilis* group) have produced food poisoning. Further studies in our laboratory have shown that the *B. subtilis* group appears notoriously as contaminants in custard fillings. For toxin determination, twenty-four-hour broth cultures of the staphylococcus were inoculated intraperitoneally into a white mouse and a guinea pig, and intravenously into a rabbit. There were no particularly noticeable effects in the mouse or guinea pig. The rabbit, although showing symptoms of toxemia at the end of three hours, recovered rapidly and was alive at the end of one month.

The staphylococcus isolated from the custard filling in question was a single strain. It produced a golden yellow pigment. The morphology was that of a typical gram-positive coccus occurring in clumps. Dextrose, maltose, saccharose, and mannitol produced acid but no gas. Lactose, raffinose, inulin, and salicin were not affected. Immunologic reactions were not used in identification.

Since the custard filling which contained the staphylococcus was heated, it seemed desirable to determine whether the organism was especially heat resistant. A broth culture in milk withstood a temperature of 70° C. (158° F.) for one-half hour. The organism probably would have been more heat resistant in the custard filling because of its thickness.

We are inclined to believe that the filling was contaminated after it was heated rather than that the organism survived the heat exposure to which it was subjected, although we think this latter quite probable.

As the symptoms appeared within three or four hours after ingestion of the cream puffs, we think the outbreak of food poisoning must be considered to be almost certainly due to a bacterial toxin or to a formed toxic substance produced by the *Staphylococcus aureus* present in the filling. We had neither facilities nor opportunity to prove this strain of *Staphylococcus aureus* to be of the food poisoning type either by monkey feeding experiments or by tests on human volunteers.

An epidemiologic investigation was made of the personnel in the bakery where the cream puffs were made. No open suppurative lesions were found. Routine cultures were taken from the nose and throat of each person employed. *Staphylococcus aureus* was found in pure culture in the throat of one of the men and showed the same morphologic, cultural, biochemical, and physiologic characteristics as the strain isolated from the custard filling of the cream puffs. It was later learned that this man had so-called grip and a sore throat at the time this culture was taken. A second specimen from this man also showed the presence of a pure culture of *Staphylococcus aureus* identical in all characteristics with the strain isolated from the first culture and also with the strain isolated from the custard filling. We think the repeated recovery of *Staphylococcus aureus* in pure culture from the throat of this man incriminates him as the original source of the etiologic agent.

Abstract of paper read at a meeting of The New Haven Medical Association, March 18, 1936.

FOLLICULAR LYMPHOBLASTOMA*

With Brief Review of Literature

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IN THE year 1925, Brill, Baehr and Rosenthal of Mount Sinai Hospital, New York, reported their observations on a condition, believed not to have been described previously, which they designated as "generalized giant lymph-follicle hyperplasia of lymph nodes and spleen."

Three patients, all women, had been considered. The first patient died following splenectomy, and no autopsy was obtained. The second and third patients were alive and in apparent good health at the time the report was made. The spleen of one was removed, but the other was treated entirely by roentgen ray with gratifying results. The material which underwent pathologic examination consisted of the two removed spleens, with two intraabdominal lymph nodes removed at the same time, and in addition cervical and axillary lymph nodes which were obtained for biopsy from the surviving patients. It was observed that all the lymph nodes throughout the body were enlarged.

In this first presentation the investigators stated that the hyperplastic process was "apparently benign." But in 1927, Baehr and Rosenthal came before the American Association of Pathologists with a further report on the same condition, which they now termed malignant lymph follicle hyperplasia. Again, in 1931, Baehr and Rosenthal, this time in collaboration with Paul Klemperer, before the same society, once more discussed the condition, stating, "The disease is a form of lymphosarcoma which deserves to be distinguished as a pathologic entity because of its characteristic pathology, its unique pathogenetic evolution and its unusual duration. It may form a connecting link between the systemic hyperplasia of the lymphatic tissue and lymphosarcomatosis." To distinguish it from other varieties of lymphosarcoma, it was proposed to term it "follicular lymphoblastoma."

CASE REPORT

Mrs. W., a white, married female, sixty-seven years of age, entered the hospital on April 18, 1935, with the chief complaint of swelling of the left side of the neck.

Four months ago, patient first noticed swelling in the left supraclavicular region which increased in size very gradually. About a week ago, she noticed swelling along the anterior edge of the left axilla. This swelling did not vary in size under any conditions. It had never been painful.

Patient had no other complaint and stated that she had always been unusually well. Her average weight was about 164, which was high for her height. In her previous medical

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Received for publication, August 3, 1936.

history the only diseases which she recalled were scarlet fever which she had had in early childhood and some indefinite fever which was called malaria. Her family history was negative as far as any glandular enlargements were concerned and also negative as regarded any ordinary chronic illnesses. Her father died at ninety-one, and her mother died at ninety-six.

Physical Examination.—

1. General Appearance: Short, stout, somewhat dark pigmentation of skin; suggested hypothyroid type.
2. Eyes: Pupils equal and reacted to light.
3. Mouth: No abnormality of mouth or teeth.
4. Throat: Negative.
5. Neck: In the left supraclavicular space toward the neck was a mass estimated to be between 3 and 4 cm. in diameter, firm, no fluctuation, not easily movable, not tender.

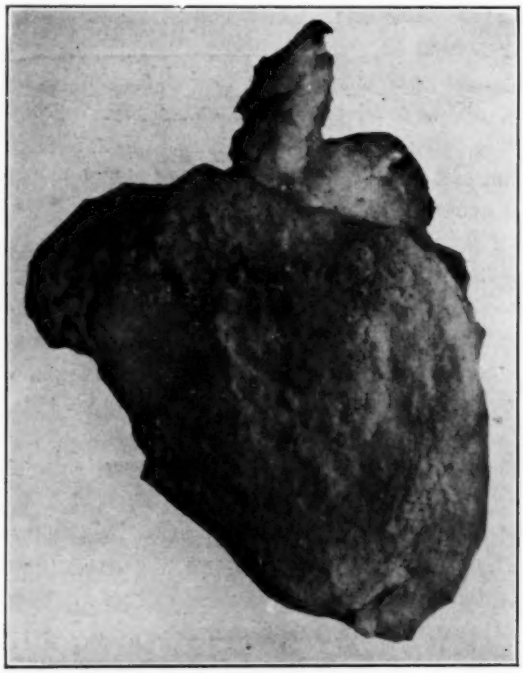

Fig. 1.—Note the prominent follicles and bulging of tissue.

6. Heart: Very moderately enlarged to the left by percussion. Left border 12 cm. from the midline. Right border was just about under the right costal margin. Rhythm regular. Rate 82. Blood pressure 188/106, left arm, sitting. Rough systolic murmur at aortic area.
7. Lungs: A few râles at the left base posteriorly.
8. Abdomen: Liver not palpable. Spleen not palpable. Abdomen otherwise negative.
9. Extremities: No axillary glands palpable. Very slight pretibial pitting in the lower portion of the leg.

Radiographic Examination of the Chest.—Films of the chest revealed no evidence of enlarged mediastinal glands. Marked hilus thickening and increased peribronchial fibrosis were seen. There were only occasional enlarged bronchial nodes seen in the hilus. Lung fields were clear of any infiltration.

Blood Counts.—Numerous blood counts were done, and the hemoglobin averaged from 14.3 gm. to 12.4 gm., and the red blood cells about 4,730,000 and white blood cells about

8,000. Polynuclears averaged about 64 per cent, lymphocytes about 35 per cent, and the eosinophiles 1 per cent. In the morphologic study of the red blood cells, an occasional microcyte was found.

Urinalysis.—

Color—yellow, clear	Sugar—negative	Indican—negative
Sp. Gr.—1.015	Protein—negative	
Reaction—acid	Acetone—negative	

Microscopic: Moderate number of squamous epithelial cells. A few large, round, epithelial cells, 1-3 W.B.C./HPF.

On re-examination on April 21, 1936, after extensive therapy, her cardiovascular condition was improved so that the pretibial pitting disappeared. The slight shortness of breath was relieved. The blood pressure fell and has never returned to the original level. The last reading on the twenty-first of April, 1936, was 140/80.

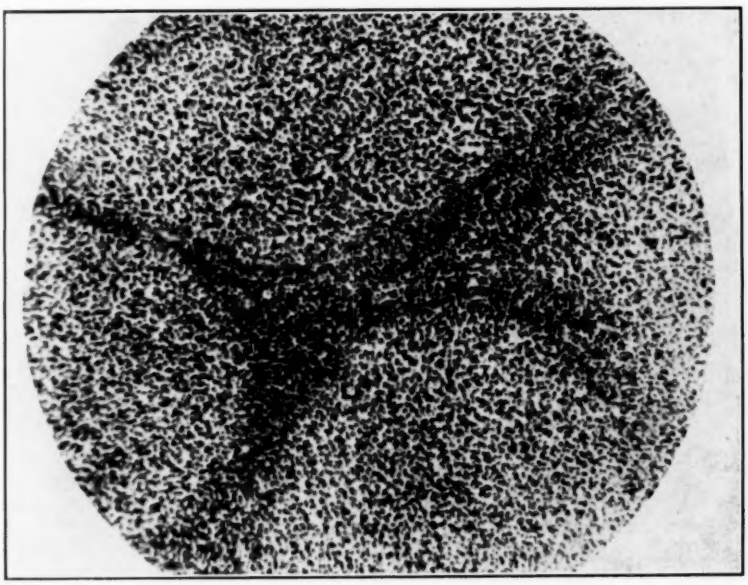

Fig. 2.—Low Power. Individual follicles occupy several microscopic fields. Notice the absence of intervening tissue.

Throughout her illness neither liver nor spleen could be palpated on frequent examinations. It was not until December, 1935, that the liver came just to the edge of the costal margin on deep inspiration, and in the right lateral recumbent position, the edge of the spleen could be palpated. It was not easily palpable in the dorsal recumbent position. It was not tender. The spleen has remained palpable ever since, although changing practically not at all in size.

The treatment consisted of deep x-ray therapy with iron and arsenic by mouth because of the slight tendency to anemia. From time to time she was given arsenic alone by mouth. The cervical and axillary lymph nodes were removed on April 18, 1935, and submitted to the laboratory for examination.

PATHOLOGIC REPORT

Cervical and Axillary Lymph Nodes

Macroscopic Examination.—Many lymph nodes were submitted for examination, which weighed collectively 180 gm., and on cut section, the capsules appeared to be intact and unin-

volved. The tissue was solid, pinkish-white in color, and bulged. The follicles were easily detected because of their prominence.

Microscopic Examination.—The individual follicles occupied several microscopic fields when viewed with low power. They were so close to each other that there was no intervening pulp visible, and the lymph sinuses were compressed and obliterated. Most of the follicles were composed of an endothelioid type of cell. The active cells with mitoses far outnumbered the pyknotic forms, which formed a dark ring around the margin of the follicle. The endothelioid type of cell was largely polygonal and contained a large amount of cytoplasm.

This lesion was considered benign by many, yet there are cases where they have recurred and finally, after many years, the condition may terminate as a lymphosarcoma. These were radiosensitive.

Diagnosis.—Follicular lymphoblastoma of the cervical and axillary lymph nodes.

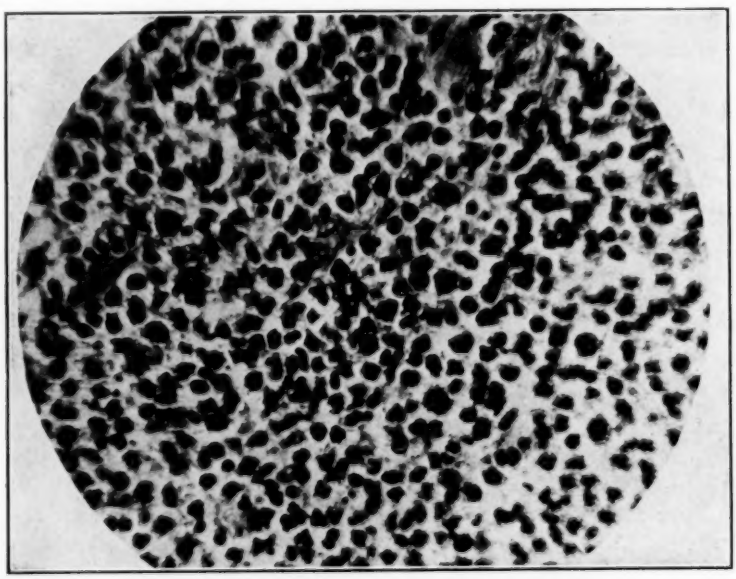

Fig. 3.—High Power. Note active cells with mitoses. The lymphocytes are at the margin of the follicles.

DISCUSSION

The salient characteristics of the condition were listed as follows:

1. Lymphadenopathy due to hyperplasia of the germinal centers of the lymph follicles.

2. Splenomegaly due chiefly to enormous enlargement of malpighian bodies, the weight of the spleen increasing up to 1,800 gm.

3. Absence of abnormal cells in the blood.

4. Absence of anemia or cachexia.

5. Tendency to development of serous effusions in the pleural and peritoneal cavities due to pressure of mediastinal or abdominal lymph nodes upon venous or lymph vessels.

6. Absence of involvement of tonsils and lymphatic apparatus of the gastrointestinal tract.

7. Tendency to lymphatic infiltration in lachrymal gland resulting in unilateral exophthalmos. Aside from the absence of anemia and cachexia, the chief differential feature, distinguishing the condition from lymphosarcoma, is its origin multicentrically throughout the body in the lymph follicles, whereas lymphosarcoma arises monocentrically and spreads by lymphatic extension.

Thus by 1933 the efforts of pathologists had placed before the medical profession a fairly clear picture of a condition previously shrouded in a mist of clinical confusion. Much obscurity still clouds this picture; etiology, pathogenesis, and clinical symptoms must still be cleared up by the slow accumulation of data supplied by individual practitioners. It is, therefore, of importance that all such data should be put on record. The present paper is an effort in that direction.

It is Klemperer's belief that in follicular lymphoblastoma we have a link between lymphadenosis and lymphosarcomatosis. "While early phases resemble aleucemic lymphadenosis, necropsy years later presents evidence of tumor formation of lymph nodes and invasion of neighboring structures, precisely as in lymphosarcomatosis. One of the cases of follicular lymphoblastoma developed a blood picture characteristic of chronic lymphatic leucemia. At necropsy, all of the lymph nodes were found to be enlarged. Other autopsied cases conformed to the anatomic picture of lymphosarcomatosis because of conspicuous aggressive tumor formation of the lymph nodes with infiltration of the neighboring organs. In the early stages of its evolution, the process is merely hyperplastic, while in the later stages it becomes atypical and aggressive. All such experiences point to a close affinity between the simple hyperplastic and the neoplastic proliferations of the lymphatic system. This is evident even in the spleen, the appearance of which has hitherto constituted one of the chief reasons for strict separation. Whenever the spleen is involved in Hodgkin's disease, the alteration is macroscopically characteristic, and can be easily differentiated from the splenic lesions observed in the hemoblastoses." In this investigator's opinion, Hodgkin's disease is essentially a granulomatous process, in which the appearance of the spleen differs fundamentally from that seen in the hemoblastoses.[4]

The diagnostic difficulties which may arise in the attempt to differentiate between Hodgkin's disease and lymphosarcoma are, in Klemperer's opinion, due to undifferentiated proliferation of the reticulum of the lymphoid tissues. The changes in the follicles he believes to be proliferation of embryonal elements which under suitable stimuli can proliferate and differentiate in the same manner as other embryonal elements placed elsewhere in the body. If we were to employ a "noncommittal term" (he suggests "progressive reticulum cell proliferation"), false interpretation of the transitions between the individual fixed groups, such as lymphosarcoma, Hodgkin's disease and so on, might be avoided.[5]

It might perhaps be well, before proceeding to a description of individual cases, to refresh our memories as to just what is meant by the term "lymphoblastoma," and estimate, even if we cannot clear away, the ambiguities which have in the past surrounded, and still surround, any consideration of this branch of the great family of entoplastic growths. Lymphoblastoma is defined by Mallory[6] as a tumor of mesodermal origin, the cells of which tend to differentiate into lymphocytes, that is, cells of the lymphocyte series. As the lymphoblast, which is the type-cell, under normal conditions may differentiate first into lymphocyte, and thence to what we term a "lymphoid cell," it is not difficult to foresee that, under pathologic conditions, such changes may take place with far greater rapidity and variation than is usual in health.

The lymphoblastoma is an infiltrative tumor, therefore its stroma is furnished by the organ or tissue that it invades. In some tumors the stroma is of the slightest, consisting chiefly of capillary blood vessels accompanied by a minimum of connective tissue, usually in the form of a reticulum. As the invaded organs enlarge, the stroma may be increased by proliferation of fibroblasts and probably by formation of new blood vessels. The lymphoblastoma probably originates from a single cell—a lymphoblast. Cells of this type occur in nearly all parts of the body, but are most common in the various lymph nodes, and in the lymph nodules of the gastrointestinal tract and spleen. On this account the lymphoblastoma may originate in various localities wherever lymphoid tissue exists, as, for example, in the cecum or spleen, but it starts most commonly in lymph nodes, especially in those of the cervical, axillary and inguinal regions, and in the mediastinum.

Discussions of the limitations of the term "lymphoblastoma" have not been lacking. Keim[7] lists no less than fifteen conditions which are so essentially similar as to justify (in his opinion at least) their inclusion under the single classification of "lymphoblastomas." The dermatologic aspect of the condition has received particular attention, and contributions regarding manifestations upon the cutaneous surface are of great interest and importance. But the similarity or identity of the skin conditions to the follicular type is still being questioned in some quarters. "True lympadenotic infiltration of the skin occurs most commonly in association with the forms of lymphatic hyperplasia, usually referred to as leukemia, lymphosarcoma, granuloma fungoides and Hodgkin's disease, as well as certain transitional forms originally described as Sternberg's leukosarcoma and kaposis lymphoderma pernicosa." Fraser[8] more than a decade ago called attention to the relationship which exists between mycosis fungoides, lymphatic leucemia and lymphosarcoma. Similar views were held by Douglas Symmers a number of years previously, for he wrote in 1918,[9] "Chronic lymphatic leukemia and its companion lesion, pseudo-leukemia, together with the familiar examples of lymphosarcoma, present many clinical and anatomic changes in common. The histologic alterations are closely akin; in fact, in many instances, they are indistinguishable one from another." Mallory's classification offers a simple way out of such difficulties as Symmers visualizes, that is, the grouping of all such lesions under the general heading of lymphoblastomas. Goeckerman and Montgomery[10] have reported a small series of cases of great interest in that they serve to illustrate how many features in common there are between certain vaguely understood skin lesions and the neoplastic growths seen in the lymph nodes and internal organs which are usually designated as lymphosarcomas. In their own words: "A clinical consideration of our two cases leaves little doubt that they belong to the group of lymphoblastomatous diseases, yet they can only with difficulty be placed definitely in subgroups under this heading because they bear none of the classic clinical and histopathologic characteristics of any one disease. In our opinion they favor the view that this entire group is closely related genetically and that it contributes to confusion rather than to clarification to insist on sharp distinctions at all times. A generic term including this entire group seems desirable. Although on etymologic

grounds there may be objections to the generic name 'lymphoblastoma' it can answer the purpose for which it is intended, at least until more is known in regard to the etiologic factors."

One hundred and fifty-six cases of lymphadenopathy were studied at Mount Sinai Hospital, New York, during a ten-year period. Of these, 80 were Hodgkin's disease; 50 were lymphosarcoma; 16 were reticulum cell sarcoma, and only 10 were diagnosed as follicular lymphoblastoma. It was upon the findings in these 10 cases that the reports from this hospital, cited earlier in this paper, were made. In 1931 Rosenthal, assisted by Harris and Kean, made still another report, this time giving special attention to the "pathologic, clinical and radiotherapeutic features. A thorough review of the literature fails to reveal any comprehensive report on the clinical manifestations or radiosensitivity of this subvariety of lymphosarcoma."

If a biopsy is done in the early stage of this disease, enormously enlarged lymphoid follicles are found which resemble markedly overgrown germinal centers. These follicles are composed of lymphoblasts and frequently show mitotic figures. The periphery of the follicle is composed of the small lymphocytes with darker staining nuclei. These morphologic and histologic findings are characteristic and are of especial interest now when so much stress is being placed upon the grading of tumors, with its bearing on radiosensitivity and prognosis.

Follicular lymphoblastoma may be mistaken for simple follicular hyperplasia. However, careful study readily differentiates the former by the presence of mitotic figures, by the large size of the follicles, and by the tendency to involvement of the fibrous capsule. The tissue does not resemble Hodgkin's disease histologically. It may be differentiated from aleucemic lymphadenosis by the remarkable follicular hyperplasia.

In a later stage of this disease, in addition to the characteristic follicular condition, some areas may show a disruption of this formation, and a rather typical picture of lymphosarcoma with infiltration of the capsule and, at times, invasion of surrounding structures. The spleen, which is usually greatly enlarged, shows a similar characteristic follicular hyperplasia.

It was the experience of these investigators that the duration of the affection was exceptionally long for disease having such characteristics. One of these patients is still alive after fourteen years. In all ten patients the first indication was enlargement of some group of nodes, cervical, axillary or inguinal. "Terminally, the body may be riddled with disease, voluminous tumor deposits occurring which give rise to pleural effusion and ascites." Four patients, all women, developed exophthalmos.

TREATMENT

While no form of treatment has yet been devised which will offer permanent relief in any of the class of interrelated diseases which we now term "lymphoblastoma," the growths have all proved radiosensitive, and clinical improvement usually follows judicious x-ray therapy. No physician is justified in holding out ultimate hope to the patient or his friends, but the long survival of some

of these patients who were systematically treated by roentgen ray exposures, gives ground for assurance that some aid may be offered. Rosenthal and his coworkers used an average dose of about 200 r., treating all the involved areas at three- or four-day intervals. "The lesions throughout the body responded readily until the terminal stage of the disease, when they became radio-resistant. In several instances, radium packs induced regression when roentgen therapy failed." It was the consensus of opinion among those who discussed the paper just quoted that surgery is of no use in the management of any of these lymph system affections. Herbert Fox of Philadelphia strove to emphasize the differences between the various clinical conditions which we have sought to link together under the common title "lymphoblastoma." He regarded this as important from the point of view of treatment. He defined lymphomatosis as a multiple tumor of lymph nodes and viscera, with or without an enlargement of the spleen. Lymphosarcoma manifests none of the characteristics of lymphomatosis; there is no reduction of neutrophiles as in leucemia; no enlargement of the spleen; no involvement of the skin or the bones; and no distinctive involvement of the lungs. In Hodgkin's disease, on the contrary, though the spleen is often large, it is not characteristically hypertrophied, while involvement of skin, bone, and lungs is very common.

It is to be hoped that further reports of pathologic findings and methods, tried out in treatment, will soon add to our knowledge of this obscure class of neoplastic growths.

REFERENCES

1. Brill, N. E., Baehr, G., and Rosenthal, N.: Generalized Giant Lymph Follicle Hyperplasia of Lymph Nodes and Spleen, J. A. M. A. **84**: 668, 1925.
2. Baehr, G., and Rosenthal, N.: Malignant Lymph Follicle Hyperplasia of Spleen and Lymph Nodes, Am. J. Path. **3**: 550, 1927.
3. Baehr, G., Klemperer, P., and Rosenthal, N.: Follicular Lymphoblastoma (Giant Follicular Hyperplasia of the Lymph Nodes and Spleen), Am. J. Path. **7**: 558, 1931.
4. Klemperer, Paul: The Spleen in Hodgkin's Disease, Lymphosarcomatosis and Leukemia, Am. J. M. Sc. **188**: 593, 1934.
5. Idem: The Relationship of the Reticulum to Diseases of the Hematopoietic System, Contrib. Med. Sc., Emanuel Libman **2**: 655, 1932.
6. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, 1914, W. B. Saunders Co., p. 326.
7. Keim, H. L.: The Lymphoblastomas, Their Interrelationships, Arch. Dermat. & Syph. **19**: 532, 1929.
8. Fraser, J. F.: Mycosis Fungiodes: Its Relation to Leukemia and Lymphosarcoma, Arch. Dermat. & Syph. **12**: 814, 1925.
9. Symmers, Douglas: Certain Unusual Lesions of the Lymphatic Apparatus, Arch. Int. Med. **21**: 237, 1918.
10. Goeckerman, W. H., and Montgomery, H.: Cutaneous Lymphoblastoma: Report of Two Unusual Cases, Arch. Dermat. & Syph. **24**: 383, 1931.
11. Rosenthal, N., Harris, Wm., and Kean, Albert: Clinical and Radio-Therapeutic Considerations of Follicular Lymphoblastoma, Am. J. Roentgenol. **29**: 95, 1933.
12. Fox, Herbert: Discussion of.[11] Ibid., p. 102.

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THE SIGNIFICANCE OF ABERRANT BASAL METABOLIC TRACINGS*

Clinical Note

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EMOTIONAL instability may at times be revealed by the spirometric tracing used in the determination of the basal metabolic rate by indirect calorimetry. The spirometer records the speed, depth, and regularity of the respiratory movements. It has been observed that irregular or sighing types of respiration are frequently found in patients with functional heart disease or with some manifestations of the psychoneuroses. The following note is written in the hope that it will call attention to the possibility of recognizing functional disorders from the inspection of spirometric tracings.

Erratic basal metabolic tracings taken by competent technicians were selected, and the corresponding patients' histories were reviewed. Basal metabolic rate determinations from 400 patients were studied, and 32 were selected as indicating a psychogenic reaction. Seventy-five per cent of the selected cases were from patients between the second and fourth decades. Females predominated. Thirty-two cases, or 8 per cent, of the records exhibit these bizarre irregularities and most of the histories indicate functional disorders. In general, the selected tracings may be divided into two types. First, those who sigh at intervals, causing exaggerated excursions of the spirometer. In the second type the slope of the tracing is wavelike with alternating crests and troughs.

Nielsen and Roth,[1] reviewing 20,000 basal metabolic tests, divide the tracings into nine types. Six of these respiratory types are found in children. One of their selected spirograms, Type G, described as "a regular inspiratory line interrupted by frequent and extremely deep inspirations," is akin to the tracings under discussion in this paper. These Type G tracings are most prevalent in females and when once established at puberty do not disappear at menopause. Ziegler and Levine,[2] determining the effects of the emotions on the basal metabolic rate, found the metabolic rate might be altered as much as 35 per cent, and there were observed changes in amplitude and rate as compared with the control tracings. Doris Baker[3] quotes Paul White as having found sighing a common symptom in women of the third and fourth decades with neurocirculatory asthenia but relatively rare in organic heart disease. She concludes that sighing respiration aids in determining the relative degree of responsibility of the heart and nervous system in the production of disability. Craig and White[4] reported on 50 cases of neurocirculatory asthenia without organic heart disease and found that 70 per cent had respiratory disturbance. Christie[5] found the anxiety neuroses produced an irregular shallow type of respiration, while the

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Received for publication, August 13, 1936.

conversion hysterias had a tendency to hyperventilation. He made his observations from spirometric tracings of 35 patients in whom a diagnosis of neurosis had been made. Clinically there was no evidence of organic disease in these cases.

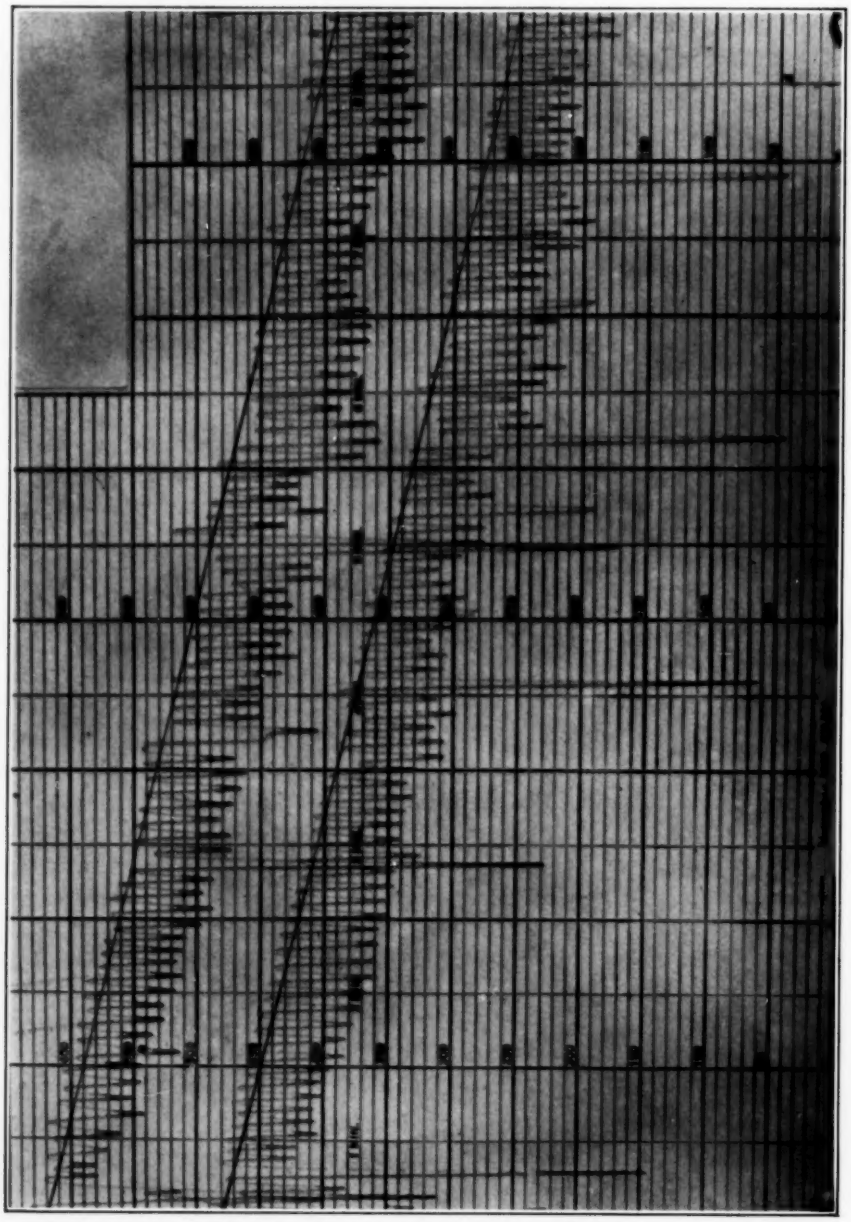

Chart 1.

CASE 1.—Man, aged thirty-five years, complained of nervousness, periodical quivering of muscles, insomnia, and restlessness. Two months before, he had experienced dyspnea on exertion, precordial distress, and a tachycardia with rate of 200. Four years earlier he had dreaded going to the hospital where he had had a bilateral inguinal herniotomy, and since then

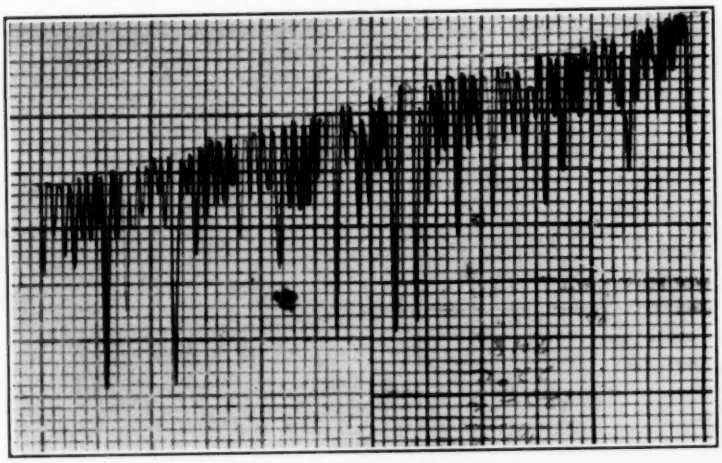

Chart 2.

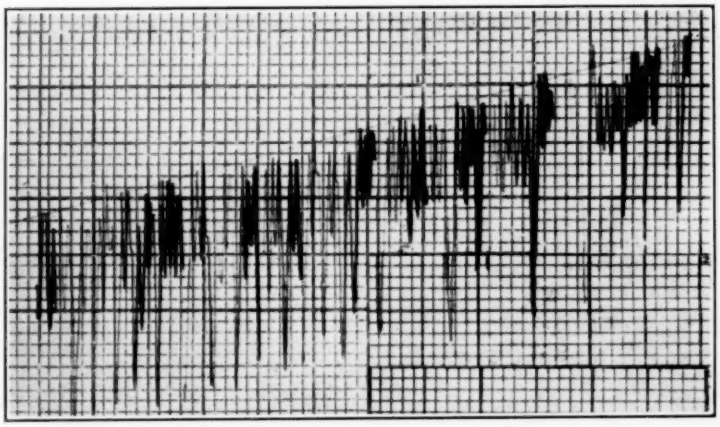

Chart 3.

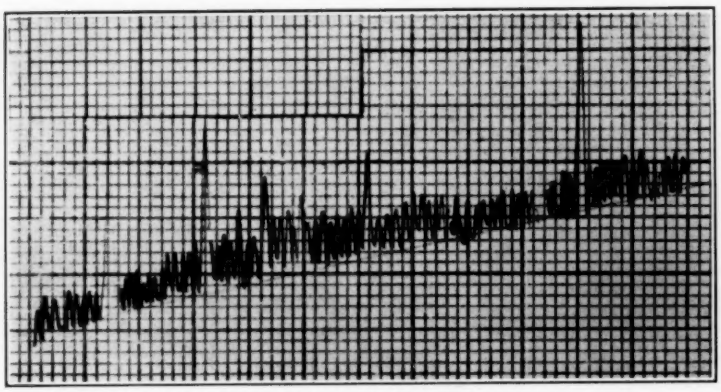

Chart 4.

he felt at times that he were dying. The laboratory examinations of urine, the blood, and Wassermann reaction, as well as complete gastrointestinal x-ray study, and basal metabolic rate of -3 per cent, were normal.

CASE 2.—A stenographer, divorced, aged thirty-two years, complained of nervousness and depression. She had periods when she felt she would suffocate and times when she was afraid she would commit suicide. She had been nervous since the birth of a child thirteen years ago and noted a sensation of abdominal pressure since an appendectomy seven years ago. On examination, indefinite tenderness of the right lower abdomen and a hypertrophic endocervicitis were found. The urine and blood were normal. The Wassermann was negative. The basal metabolic rate was +10 per cent.

CASE 3.—Woman, aged twenty-seven, single, complained of nervousness, insomnia, a choking sensation and feeling of enlargement of the neck for five years. She had outbursts of weeping, and for the last year there had been palpitation of the heart after excitement or exertion. Examination revealed an intelligent woman who was nervous, without exophthalmus and with a thyroid gland whose right lobe was hard, nodular, and four times normal size. The heart was found to be normal. Her hands were cold and moist. The laboratory reported a negative blood Wassermann, a normal blood count and urine. The basal metabolic rate was +22 per cent on June 16, 1933. On July 19, 1933, the patient had a thyroidectomy and the pathologic diagnosis was cystic adenoma. The patient returned on Aug. 7, 1933, complaining of regurgitation immediately after eating. A gastrointestinal x-ray study revealed no abnormalities of the esophagus, stomach, or duodenum. The appendix, however, was found to be tender, and after forty-eight hours was visualized by the roentgenologist, who considered it to be diseased. The basal metabolic rate checked on Oct. 30, 1933, was +10 per cent, and shortly afterward an appendectomy was performed. Two years later the patient was still regurgitating her food and complaining of palpitation when nervous.

A review of these tracings demonstrates a respiratory pattern of irregular breathing and deep sighs. The usual spirometer tracing does not have these irregularities. It is of particular interest to follow the two graphs of Case 3. The first tracing shows evident lack of balance of the respiratory mechanism and reflects the high degree of anxiety due to the patient's emotional reaction to her moderate physical disability. The second tracing, taken three months later, still shows sighing respiration, but as indicated by her subsequent improvement, this is less extreme than it was.

It is profitable to inspect the actual tracing rather than accept only the calculated rate of metabolism. A bizarre pattern suggests the possibility of an environmental or psychogenic component in the patient's reaction. The evaluation of this too frequently neglected factor will in turn give a more exact insight into the patient's disease.

REFERENCES

1. Nielsen, J. M., and Roth, P.: Clinical Spirography; Spirograms and Their Significance, Arch. Int. Med. **43**: 132, 1929.
2. Ziegler, L. M., and Levine, B. S.: Influence of Emotional Reactions on Basal Metabolism, Am. J. M. Sc. **169**: 68, 1925.
3. Baker, D. M.: Sighing Respiration as Symptom, Lancet **1**: 174, 1934.
4. Craig, H. R., and White, P. D.: Etiology and Symptoms of Neurocirculatory Asthenia; Analysis of 100 Cases With Comments on Prognosis and Treatment, Arch. Int. Med. **53**: 633, 1934.
5. Christie, R. V.: Some Types of Respiration in Neuroses, Quart. J. Med. **4**: 427, 1935.

A STUDY OF THE RELATION OF RICKETS TO ANEMIA*

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THE association of anemia with rickets in infancy has been a disputed question among clinicians for many years. Some observers regard anemia as a symptom of rickets and feel that a causative factor is to be found in the changes which occur in the bone marrow in this disease. Hess[1] states that Marfan is one of the foremost of such observers. On the other hand, many believe the anemia in rickets is produced by complicating factors such as poor diet and infection. Findlay,[2] in a study of 30 cases of active rickets, found anemia to be the result of complications in most of the group and discovered that many of his rachitic infants had normal or elevated hemoglobin and red blood cell counts. Griffith and Mitchell[3] state that the hemoglobin of the blood is reduced in severe cases of rickets. Royster[4] has remarked about the frequent association of rickets with chronic nutritional disorders such as lead to secondary anemia. Hess[5] feels that anemia is not a characteristic symptom of rickets but states that, as the disorder advances, it is generally accompanied by anemia of some degree. We have studied a group of thirty infants and children, ranging in age from six months to three years, who were admitted to the State of Wisconsin General Hospital with a diagnosis of active rickets. Table I contains a list of these cases divided into the uncomplicated and complicated groups. Of the whole series of cases, suffering from mild to severe acute rickets, 16 or 53 per cent had normal hemoglobin readings and red blood cell counts. Fourteen or 47 per cent had readings of 60 per cent hemoglobin or lower. Eight of the cases showing low hemoglobin readings were complicated by dietary deficiencies or infections. Eleven cases in the entire group were complicated by dietary deficiencies, infections or anomalies such as cleft palate. Eight of the eleven complicated cases showed anemia whereas only six of the nineteen uncomplicated cases revealed a reduction in hemoglobin.

In an attempt to throw some light on the relationship of anemia to rickets, experiments were undertaken to try to produce the picture of rickets with anemia in the chick. This work consisted of the production of severe rickets in the chick and a comparison of the hemoglobin content of the blood of the rachitic with that of the normal chick. Analyses of the livers of normal and rachitic chicks for their total and available iron contents were also made.

EXPERIMENTAL

One-day-old white leghorn chicks were used in these studies. They were housed in the usual manner in heated cages on two mesh to the inch wire screens.

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Received for publication, September 18, 1936.

TABLE I

BLOOD STUDIES AND X-RAY FINDINGS OF 30 INFANTS AND CHILDREN SUFFERING FROM UNCOMPLICATED AND COMPLICATED RICKETS

AGE	HB* %	R.B.C.	X-RAY FINDINGS	CA	P	REMARKS
Uncomplicated Rickets (Active)						
18 mo.	73	5,000,000	Severe	–	–	
16 mo.	45	4,000,000	Moderate	9.4	3.1	
21 mo.	85	6,240,000	Severe	7.8	5.68	
18 mo.	74	4,990,000	Severe	13.7	2.6	
11 mo.	55	4,270,000	Healing	–	–	
12 mo.	55	4,640,000	Severe	7.8	–	
6 mo.	72	3,800,000	Moderate	–	–	
22 mo.	69	4,000,000	Severe fracture	–	–	
10 mo.	52	4,650,000	Severe	9.5	7.0	
30 mo.	94	4,900,000	Moderate	12.7	4.63	
25 mo.	85	5,420,000	Moderate	–	–	
24 mo.	50	4,300,000	Moderate	–	–	
24 mo.	75	4,630,000	Moderate	12.3	3.87	
36 mo.	65	4,800,000	Severe	7.18	4.00	
24 mo.	78	4,500,000	Severe	9.31	3.55	
19 mo.	75	4,800,000	Moderate	10.6	3.8	
19 mo.	65	4,300,000	Moderate	–	–	
11 mo.	65	4,410,000	Mild	11.7	5.5	
11 mo.	50	4,160,000	Severe	9.3	2.9	
Complicated Rickets (Active)						
7½ mo.	55	4,100,000	Early mild	–	–	Poor diet
7½ mo.	59	3,630,000	Mild	–	–	Cleft palate, diet poor
8 mo.	60	4,670,000	Severe	–	–	Spasmophilia
8 mo.	42	3,810,000	Severe	–	–	Upper respiratory infection and otitis media
12 mo.	18	3,880,000	Severe	–	–	Poor diet. Nutritional anemia
17 mo.	35	5,000,000	Moderate	–	–	Poor diet
12 mo.	55	4,900,000	Healing	–	–	Acute suppurative cervical adenitis
12 mo.	70	4,100,000	Mild	–	–	Cleft palate
9 mo.	50	4,600,000	Severe	7.74	–	Spasmophilia-upper resp. infection and otitis media
18 mo.	65	4,410,000	Moderate	5.59	3.4	Spasmophilia and upper resp. infection
30 mo.	70	4,100,000	Moderate	–	–	Cretinism

*Hemoglobin readings by Tallquist Method.

One group consisting of 15 one-day-old chicks was placed on a basal ration recommended by Hart, Kline and Keenan[6] which consists of:

Yellow corn	59 parts
Wheat middlings	25 parts
Crude casein	12 parts
Ca carbonate	1 part
Ca phosphate	1 part
Dried yeast (A. B.)	1 part
Sodium chloride	1 part

Chicks fed on this ration developed severe rickets in three to four weeks. The control group of 15 one-day-old chicks was placed on the same ration supplemented with 1 per cent of cod liver oil. Hemoglobin determinations, using a modification of the Newcomber method recommended by Schultze and Elvehjem,[7] were made at weekly intervals beginning in the second week. The average results in grams of hemoglobin per 100 c.c. of blood are shown in Table II. It will be noted that the greatest difference in hemoglobin occurred in the fifth week, at

which time the average readings for the normal and rachitic groups were 8.60 gm. and 7.64 gm., respectively. The difference of 0.96 gm. was not regarded as significant. This experiment was repeated, using groups of 10 chicks each and hemoglobin readings were made in the second and fifth weeks. The average results which were almost identical are recorded in Table II.

TABLE II

HEMOGLOBIN CONTENT OF THE BLOOD OF RACHITIC AND NORMAL CHICKS
AVERAGE READINGS IN GRAMS OF HB/100 C.C. OF BLOOD

AGE IN WEEKS	PART I		PART II	
	NORMAL (15 CHICKS)	RACHITIC (15 CHICKS)	NORMAL (10 CHICKS)	RACHITIC (10 CHICKS)
2	8.45	8.45	8.54	8.42
3	8.02	7.64		
4	8.00	7.98		
5	8.60	7.64	8.44	8.47

In order to determine the amount of total and available iron in the livers of the normal and rachitic chicks, the experiment was repeated, using the same method as described above. Each group placed on the experiment contained 10 to 15 one-day-old, white leghorn chicks. Beginning in the fourth week, after signs of well-marked rickets had developed, several chicks were taken from each group, killed by decapitation, and the livers removed and analyzed for the total and available iron content. The livers were washed thoroughly in distilled water

TABLE III

TOTAL AND AVAILABLE IRON CONTENT OF LIVERS FROM RACHITIC AND NORMAL CHICKS

NORMAL CHICK NO.	AGE IN WEEKS	WEIGHT IN GRAMS	WEIGHT OF FRESH LIVER	AVAILABLE IRON IN MG./GRAM OF LIVER		TOTAL IRON IN MG./GRAM DRY LIVER	PERCENTAGE OF AVAILABLE IRON
				FRESH	DRY		
			NORMAL				
4173	4	208	6.1447	0.029	0.117	0.25	46
4174	4	209	6.8688	0.0275	0.110	0.179	61
4141	5	293	8.1043	0.0275	0.110	0.23	48
4142	5	294	9.1507	0.028	0.112	0.282	40
4144	6	272	8.8043	0.0215	0.0855	0.187	45
4149	6	270	11.4964	0.035	0.140	0.22	64
4146	8	460	14.5161	0.0242	0.0968	0.20	48
4147	8	367	9.0451	0.039	0.156	0.316	49
4150	8	385	11.3351	0.029	0.116	0.232	50
			Average	0.0291	0.116	0.232	50
RACHITIC CHICK NO.			RACHITIC				
4131	4	130	2.3517	0.0348	0.134	0.24	56
4133	4	120	3.7635	0.0382	0.157	0.33	46
3592	4 (D)	130	4.1789	–	–	0.50	–
4132	6	152	4.9358	0.050	0.20	0.40	50
4135	6	154	5.2232	0.041	0.164	0.398	42
4139	6	175	3.6218	0.035	0.140	0.30	47
4140	6	174	1.2640	0.032	0.128	0.255	50
4136	8	235	6.0869	0.027	0.108	0.246	44
4172	8	170	3.9194	0.047	0.188	0.40	47
4138	8	185	4.7272	0.038	0.152	0.266	57
			Average	0.0381	0.152	0.330	48.5

and that portion used for the determination of total iron dried in a 100° oven for twenty-four hours. The available iron was determined by the bipyridine method of Kohler, Elvehjem and Hart.[8] The total iron was determined by the thiocyanate method, or the bipyridine method for total iron recommended by Lintzel.[9] The results for the normal and rachitic groups are recorded in Table III.

From the results obtained, by the methods used in this experiment, it is apparent that we have been unable to show a reduction of the blood hemoglobin in the rachitic chick, or demonstrate a significant difference in the total and available iron content of the normal and rachitic chick livers.

In order to demonstrate, if possible, a relationship between the absorption and storage of iron and the disturbance of calcium and phosphorus metabolism in the rachitic chick, the following experiment was performed. One group of twenty-one-day-old white leghorn chicks was placed on the rachitogenic ration described above. Another group of twenty-one-day-old chicks was placed on this basal ration supplemented with 1 per cent of cod liver oil. Hemoglobin readings were made in the sixth week and average readings for the twenty chicks in each group were 9.59 gm. per 100 c.c. for the normal controls and 8.82 gm. per 100 c.c. for the rachitic chicks. At the age of six weeks, when a severe degree of rickets had developed in the chicks on the basal ration, five chicks from each group were killed and the livers analyzed for their total and available iron content. The remaining chicks of both groups were then given the basal rations supplemented with 0.1 per cent of iron in the form of ferric sulphate. At the end of three days, seven days, and fourteen days, five chicks from each group were killed and the livers analyzed for their total iron content. The results of this experiment are recorded in Table IV. The available iron was not done on

TABLE IV

THE EFFECT ON THE IRON CONTENT OF RACHITIC AND NORMAL CHICK LIVERS PRODUCED BY FEEDING IRON

AGE WHEN KILLED	GROUPS	AVERAGE HB IN GRAMS/100 C.C. (20 CHICKS)	AVERAGE TOTAL IRON (LIVER) MG./GM. DRY LIVER	AVERAGE AVAILABLE IRON (LIVER)	
				MG./GM. FRESH LIVER	MG./GM. DRY LIVER
Six weeks No iron	Normal	9.59	0.332	0.03	0.12
	Rachitic	8.82	0.334	0.038	0.152
Six weeks and 3 days 0.1% added iron for 3 days	Normal		0.30		
	Rachitic		0.42		
Seven weeks 0.1% added iron for 7 days	Normal		0.36		
	Rachitic		0.391		
Eight weeks 0.1% added iron for 14 days	Normal		0.47		
	Rachitic		0.498		

the groups killed after the age of six weeks, because it was felt that the total iron content of the livers gave us enough information about the absorption and storage of the added iron. It will be noted from a study of Table IV that the average total and available liver iron varied very little in the normal and

rachitic groups. With the exception of the normal group killed at the age of six weeks and three days, the absorption and storage of iron, as indicated by the total liver iron, was increased in both normal and rachitic groups. However, there was no significant variation in the total iron content of the livers, after seven and fourteen days of iron supplement, as indicated by the figures for the normal and rachitic chicks killed at the age of seven and eight weeks. These results, obtained by the experimental methods described, suggest that there is no interference with the absorption and storage of iron in the rachitic chick.

DISCUSSION

Although these experiments indicate that anemia does not occur as a symptom of rickets in the chick, the question arises as to whether or not the rachitic picture in the chick may be compared with that of the human subject. Studies of the blood of chicks rendered rachitic on the rations used in these experiments reveals alterations in the blood chemistry similar to those encountered in infantile rickets. These changes consist of a reduction of serum calcium and phosphorus. Studies of the bone reveal a reduction in the calcium and phosphorus of the bone when rickets is produced on these rations. However, the development of severe rickets in the chick is very rapid and becomes quite marked in the third and fourth weeks of life. When we consider the brief life span of the chick, as compared to that of the human subject, it is conceivable that the period of the development of infantile rickets, usually during the first six months to one year of life, may well be compared to the period of development of rickets in the chick. There are, of course, many unknown factors which may play a part in the production of rickets in the child, while in the experimental work with the chick, we were dealing with a true vitamin D deficiency.

Maughan[10] has recently reported the development of hemoglobinemia in the young rachitic chick. Although no information as to the type of diet used in his studies is given, severe rickets was produced in seven weeks. Hemoglobin readings, as determined by the Hellige solid plane hemometer at the age of seven weeks, were from 2 to 4 gm. below the level of the normal controls. Irradiation of the rachitic chicks without other therapy produced a prompt rise in the blood hemoglobin to normal levels. By the methods used in our experiments, we have been unable to demonstrate such a reduction in hemoglobin.

Shelling and Josephs,[11] in 1934, performed some interesting experiments with normal rats. They found that rats fed a diet high in calcium and low in phosphorus had a lower percentage of hemoglobin in the blood than did those fed on a normal diet. They showed that iron retention, as measured by the total iron content of the desiccated animal, was much lower in rats fed high calcium levels. They also demonstrated that iron retention was increased by the administration of viosterol in rats fed high phosphorus levels but was not increased in animals fed high calcium. Although no conclusions were drawn from these experiments they suggest a definite relationship between disturbances of calcium and phosphorus metabolism and anemia. The theory was advanced that the iron was carried down in the intestinal tract either before absorption had taken place or during the process of excretion.

It is entirely possible that a high calcium intake or a high calcium-phosphorus ratio may retard iron assimilation, and we are now extending our studies to this question. However, rickets in infants is most likely due to low calcium or vitamin D intake rather than a disturbed calcium-phosphorus balance. Repeatedly we have seen anemia in rickets fail to respond to the administration of cod liver oil or viosterol without additional measures. The administration of iron salts to rachitic infants, without supplementing the diet with cod liver oil, raises the hemoglobin levels satisfactorily.

From the clinical evidence and the experimental results with the chick, it may be concluded that anemia is not a symptom of rickets but is probably secondary, in the infant, to complicating factors such as nutritional deficiency or infections. In very advanced, severe rickets, the poor appetite for supplementary foods and lowered resistance to infection are probably the greatest factors in the production of anemia.

SUMMARY

1. The problem of anemia in rickets is briefly reviewed from a clinical viewpoint.

2. We have been unable to demonstrate a significant difference in the hemoglobin content of the blood of normal and rachitic chicks. The hemoglobin readings were made between the ages of two to six weeks.

3. Analyses of the livers of normal and rachitic chicks revealed no significant difference in the total and available iron content.

4. In the rachitic chick, the abnormal calcium and phosphorus metabolism had no effect on the absorption and storage of iron so far as could be demonstrated by the methods used in our experiments.

The authors wish to express their appreciation for the suggestions and encouragement of Dr. C. A. Elvehjem of the Department of Agricultural Chemistry and Dr. J. E. Gonce, Jr., of the Department of Pediatrics.

REFERENCES

1. Hess, A. B.: Rickets, Including Osteomalacia and Tetany, Philadelphia, 1929, Lea and Febiger, p. 245.
2. Findlay, L.: The Blood in Rickets, Lancet **1**: 1164, 1909.
3. Griffith, J. P. C., and Mitchell, A. G.: The Diseases of Infants and Children, Philadelphia and London, 1933, W. B. Saunders and Co., p. 451.
4. Royster, L. T.: Clinical Pediatrics **10**: New York and London, 1927, D. Appleton-Century Co., p. 241.
5. Hess, A. F.: Abt's Pediatrics **2**: Philadelphia and London, 1923, W. B. Saunders and Co., p. 936.
6. Hart, E. B., Kline, O. L., and Keenan, J. A.: A Ration for the Production of Rickets in Chicks, Science **73**: 710, 1931.
7. Schultze, M. O., Elvehjem, C. A., and Hart, E. B.: An Improved Method for the Determination of Hemoglobin in Chicken Blood, J. Biol. Chem. **105**: 253, 1934.
8. Kohler, G. O., Elvehjem, C. A., and Hart, E. B.: Modification of the Bipyridine Method for Available Iron, J. Biol. Chem. **113**: 49, 1936.
9. Lintzel, Wolfgang: Zur Methodik der Mikrobestimmung des Eisens in Biologischem Material, Ztschr. ges. Exper. Med. **86**: 269, 1933.
10. Maughan, G. H.: Hemoglobin Studies in Rachitic Chickens; the Effect of Ultra-Violet Irradiation, Proc. Soc. Exper. Biol. & Med. **32**: 389, 1934.
11. Shelling, D. H., and Josephs, Hugh: Calcium and Phosphorus Studies: X, The Effects of Variation of Calcium, Phosphorus and Vitamin D in Diet on Iron Retention in Rats, Bull. Johns Hopkins Hosp. **55**: 309, 1934.

LABORATORY METHODS

THE VALUE OF THE BLOOD XANTHOPROTEIN REACTION IN DIAGNOSIS AND PROGNOSIS*

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INTRODUCTION

IN THE search for a simple and reliable test which would enable the clinician to determine or exclude quickly renal insufficiency as cause of coma, it was suggested to me by Jaffé to try the xanthoprotein reaction which Becher had found to be highly satisfactory.

The rôle of the phenols in the xanthoprotein reaction was first pointed out by Salkowski. Morner found that it was positive with tyrosine and tryptophane. Rakestraw (1923) reported from 1.7 to 3.2 mg. phenols per cubic centimeter in the blood of normal individuals. Theis and Benedict (1918) found from 1.9 to 8 mg. of phenols in 100 c.c. of blood in a variety of pathologic conditions, while Becher, Litzner, and Taglich (1925 and 1926) give the normal phenol content of the blood as 0.04 to 0.12 mg. per 100 c.c.

The aromatic substances of the blood are derived from the oxidation of tryptophane, tyrosine, and phenyl alanine in the body metabolism or in putrefaction in the intestine. Under pathologic conditions there is a definite increase of these substances in the blood. Renal insufficiency, liver damage with changes in the deamidization function and increased protein destruction, metabolic disorders and neoplasm with a large amount of tissue destruction will increase the amount of phenols in the blood.

The study of the aromatic substances by the xanthoprotein reaction in the blood in normal and pathologic individuals has been carried out very thoroughly by Becher with his associates. In 1924 he reported an increase of the aromatic substances, an ether soluble group comprising phenols, cresols, aromatic oxyacids, and indoxyl, and another nonether soluble group containing the amino acids, tyrosine-phenyl-alanine and tryptophane occurring in renal insufficiency. Other conditions, liver damage, cardiac decompensation, infections, ileus, pernicious anemia, and cadaver blood gave an increased xanthoprotein reaction.

The intensity of the reaction was measured by comparing it with a standard aqueous solution of 0.03874 per cent potassium dichromate in an Autenrieth colorimeter.

More accurate methods were devised to determine quantitatively the amount of phenol present. Becher, Litzner, and Taglich (1926) reported a

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Received for publication, April 23, 1936.

colorimetric procedure, utilizing the Millon reaction and a comparing solution of phenol cresol. They determined free and bound phenols and found values up to 3.5 mg. per cent in different pathologic conditions. Kammer (1933) determined the phenol content by using a tyrosine solution as a standard in a colorimeter and found values up to 5.55 mg. per cent.

However, Becher (1924) pointed out that it was not necessary to gauge the strength of the reaction to determine the presence of renal insufficiency. A definitely positive reaction gave a strong golden color which was unmistakable and could be easily recognized.

Becher and Koch (1925) pointed out that uremia was less dependent on the retention of nitrogenous products, that phenol poisoning resembled true uremia and that the aromatic substances played an important rôle in the pathogenesis of uremia.

Becher (1925) found a slight or no increase of the phenols in the blood of patients with acute nephritis, although there may be a considerable increase in the urea and uric acid. Granular atrophy of the kidney produced a definite increase of the phenols, while true uremia presented the highest increase.

The value of the test for prognosis and as an aid in differential diagnosis especially in comatose conditions was emphasized by Boeminghaus (1926), Tonietti (1929), Scherk (1927), Hoesch (1931), Zamyslowa (1931), and others.

A large literature has evolved from the work of Kammer, Widenhorn, Gromer, Goldman and Burniewicz, Inoue, Rathery and Waitz, Tschilow, Melly, and Wuhrman, who have found high values of the xanthoprotein reaction in uremia, chronic nephritis, arteriosclerotic kidney, with elevated values in creosote poisoning, enteritis, volvulus of intestines, thyrotoxicosis, cardiac decompensation, liver cirrhosis and atrophy, pneumonia, diabetes mellitus, carcinoma, brain tumor, pernicious anemia, lung involvements, blood dyscrasias, agonal states, cadavers, and after large doses of salicylates. Low values were found in acute nephritis.

Of special interest is the question as to what effect phenol poisoning has on the blood xanthoprotein reaction. Smith (1933) reported a study of phenols in rabbits. He found approximately 0.5 mg. per cent of phenols in different tissues of the normal animal. Free phenols, ranging from 7 to 26 mg. per cent, were found in various tissues of animals after the administration of lethal doses of phenol and orthocresol. Becher, Litzner and Taglich (1926) found 1.4 per cent mg. free and 2.1 mg. per cent bound phenol in a case of severe lysol poisoning. Incidentally, this was the highest value obtained in a study of phenols in a variety of conditions. Goldman and Burniewicz (1928) studied a case of creosote poisoning and obtained a blood xanthoprotein reaction of 45 per cent. Such findings make it necessary to include poisonings from various phenol compounds with conditions giving a strong blood xanthoprotein reaction.

Technic of the Xanthoprotein Reaction.—

The xanthoprotein reaction was performed as described by Becher (1924).

1. Whole blood, plasma, serum, body fluids, i.e., pericardial, pleural or peritoneal, or cerebrospinal fluid may be used. It is wise in the case of whole blood to secure 10 c.c. and centrifuge.

2. Add 5 c.c. of 20 per cent aqueous solution of trichloracetic acid to 5 c.c. of the plasma, serum, or body fluid. Mix thoroughly and allow to stand for ten minutes. Filter.

3. Add 0.5 c.c. of concentrated nitric acid to 2 c.c. of clear filtrate obtained from Procedure 2.

4. Heat to boiling for one-half minute. Note: In cases that are markedly positive, a yellowish color is obtained at this point in the procedure.

5. Allow to cool and then add 1.5 c.c. of a 33 per cent aqueous solution of sodium hydroxide.

6. The reaction is best examined in direct daylight. A positive test gives a golden yellow color.

Investigators have compared the xanthoprotein reaction with standards to determine different intensities of the test. Becher (1924) used a 0.03874 per cent aqueous solution of potassium dichromate in an Autenrieth colorimeter.

Different dilutions of the potassium dichromate solution were placed in Wassermann tubes. The undiluted solution was called 1, a 90 per cent solution was called 2, and so on to a 10 per cent solution, which was labeled 10. The xanthoprotein reaction to be gauged was placed in a similar Wassermann tube and compared with the different dilutions of the standard.

Many of the xanthoprotein reactions gave a color of a much greater intensity than the Standard 1. This is the usual case in uremia and marked renal insufficiency.

Observations.—The data for this study came from patients in the wards of the Cook County Hospital or from deceased patients examined in the Department of Pathology of the same hospital. A total of 210 cases were studied and were classified as follows:

		TOTAL CASES
1. Cases admitted in coma		26
2. Cases with xanthoprotein reaction of 1 or stronger		11
3. Cases giving a xanthoprotein reaction from 2 to 10 They were further subdivided on the basis of survival and the urea nitrogen findings		68
A. High urea nitrogen and died	8	
B. Low urea nitrogen and died	16	
C. High urea nitrogen and survived	5	
D. Low urea nitrogen and survived	39	
Total cases	68	
4. Cases excluding chronic nephritis and coma without blood chemistry determinations		57
5. Cases from which blood was obtained at autopsy		35
6. Cases from which blood and other body fluids were obtained at the postmortem examination		13
Total cases		210

Coma Cases.—Twenty-six cases of patients admitted in coma or stuporous states are listed in Table I. In a few cases indican determinations were made. Four patients gave positive reactions to the xanthoprotein test. Blood chemistry was determined in three of the patients, and in all these patients there was a marked elevation of urea nitrogen. The indican test, although obtained in all four cases, was elevated to a uremic level in only two cases (9 and 18). All the patients died in one to ten days with uremia.

TABLE I

COMA CASES

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL CONDITION	BLOOD				RESULT	FINAL DIAGNOSIS
			XANTH.	INDICAN MG. %	UREA N. MG. %	CREATININE MG. %		
1. C-70-M	Hypertensive heart disease	Irrational Stuporous	6	0.25	37	2.3	Died	Hypertensive heart disease
2. W-76-M	Organic heart disease and exposure	Semicomatose	6	–	15	–	Died	Cerebral accident
3. C-65-M	Uremia	Coma	10	0.05	18	–	Died	Postmortem ecc. hypt. of heart
4. C-50-M	Chr. nephritis with uremia	Coma	8	–	8	–	Died	Postmortem mod. eccentric hypertrophy of heart
5. W-61-M	Syphilitic thrombosis	Stuporous Aphasic	7	–	15	–	Discharged	Cerebral hemorrhage
6. W-?-M	Hypertension with cerebral accident	Coma	7	–	15	–	Died	Cerebral hemorrhage
7. W-66-M	Hypertension and nephrosclerosis	Irrational Unable to speak	5	–	24	1.8	Died	Syphilitic cerebral thrombosis
8. C-37-F	Uremia, Vincent's angina	Coma	5	1.0	80	6.6	Died	Acute tonsillitis also marked dehydration
9. W-56-M	Uremia	Semistuporous	1	4.0	160	10.0	Died	Uremia, lived 1 day
10. C-55-F	G. I. Malignancy, uremia	Responded very poorly	1	1.5	73	6.6	Died	Chr. nephritis Uremia, lived 10 days
11. C-54-F	Hypertensive heart disease	Coma	1	2.0	126	19.0	Died	Postmortem Ca. of cervix with obstruction of ureters with uremia. Lived 3 days
12. C-60-M	Diabetic coma	Semiconscious and irrational	3	–	29	2.0	Died	Diabetic coma
13. W-68-M	Generalized arteriosclerosis	Irrational	7	–	18	–	Discharged	Coronary sclerosis
14. W-35-M	Alcoholic coma	Coma	7	–	13	–	Discharged	Alcoholism with coma
15. C-64-M	Uremia	Coma	9	0.15	100	9.5	Died	Coronary thrombosis
16. C-105-M	Hypertrophied prostate	Senile	8	2.00	55	4.0	Died	Prostatic hypertrophy and alkaline cystitis
17. W-62-F	Hypertension uremia	Semistuporous	9	0.05	48	2.8	Died	Postmortem mod. ecc. hypt. of heart
18. C-53-M	Carcinoma of prostate	Disoriented	1	3.0	–	–	Died	Ca. of prostate and arteriosclerosis with uremia. Lived 4 days

TABLE I—CONT'D

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL CONDITION	BLOOD				RESULT	FINAL DIAGNOSIS
			XANTH.	INDICAN MG. %	UREA N. MG. %	CREATININE MG. %		
19. W-59-M	Uremia and nephrosclerosis	Coma	9	0.15	35	2.3	Died	Hemiplegia encephalomalacia
20. C-31-M	Epileptiform seizures	Coma	6	–	–	–	Released	Epilepsy
21. W-45-M	Subacute bacterial endocarditis	Coma	7	0.05	16	–	Died	Cerebral hemorrhage
22. W-55-M	Acute alcoholism	Coma	7	0.45	–	–	Discharged	Alcoholic coma
23. W-50-M	Malignant endocarditis	Coma	5	–	28	2.0	Died	Postmortem malignant endocarditis
24. C-55-M	Bronchopneumonia	Semistuporous	6	–	16	–	Discharged	Bronchopneumonia
25. W-44-M	Epilepsy	Convulsions	9	–	–	–	Discharged	Epilepsy, traumatic
26. W-21-M	Alcoholism	Irrational	8	–	–	–	Died	Acute alcoholism

Of the remaining 22 patients, 7 recovered and were discharged. The xanthoprotein reactions were low. The other 17 patients died, none of renal insufficiency, although upon admission 7 were diagnosed as such. The urea nitrogen was elevated in 8 patients. The xanthoprotein reaction ranged from 3 to 10.

The results confirm the findings of other investigators. All patients who presented a xanthoprotein value of 1 died of renal insufficiency. No definite conclusions can be established as to the diagnosis or prognosis in patients with lower xanthoprotein values.

The findings, which agree with those reported by Becher (1930), show that the xanthoprotein reaction was more sensitive than the indican test. In only 2 of the 4 patients with renal insufficiency did the indican content of the blood approach the uremic level.

Unfortunately permission for postmortem examination could be obtained in only 5 patients.

Cases With High Xanthoprotein Values.—Table II lists 11 patients with renal insufficiency who survived from one to ten days after the xanthoprotein reaction was obtained, and all died in uremia. The urea nitrogen was determined in 10 cases and ranged from 42.49 to 206 mg. per cent. The xanthoprotein test gave values of one in all cases.

The author concludes from these findings that a patient with a xanthoprotein reaction of one presents a case of marked renal insufficiency. The longest period of survival in the coma group and this series of cases was ten days.

TABLE II

HIGH XANTHOPROTEIN REACTIONS—PATIENTS DIED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD				DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
1. C-60-M	Carcinoma of prostate	Developed pericardial friction rub	106	8.0	1	2.0	5	Postmortem—Adenocarcinoma of prostate with obstruction of urinary bladder and uremia
2. W-47-M	Chronic nephritis	Developed comatose state	105	8.5	1	1.5	½	Chronic nephritis with uremia
3. C-46-F	Arteriosclerosis; hypertension		206	8.4	1	3.0	1	Postmortem—Malignant nephrosclerosis with uremia
4. W-43-M	Asthmatic bronchitis; nephritis, pre-uremia	Developed convulsive state. Spinal Wass. 4+	86	10.0	1	1.0	5	Syphilitic meningitis; uremia
5. C-32-F	Chronic nephritis with hypertension		65	4.2	1	7.1	2	Postmortem—Malignant nephrosclerosis. Fibrinous pericarditis with uremia
6. C-45-M	Hypertensive heart disease	Decompensated	191	14.0	1	3.0	6	Postmortem—Ecc. hypertrophy of heart. Arteriosclerotic kidneys with uremia
7. C-35-M	Hypertensive heart disease	Decompensated. Pericardial friction rub	42	2.4	1	2.9	4	Postmortem—Chronic nephritis with uremia
8. W-56-M	Uremia, hemiplegia	Semistuporous	160	10.0	1	4.0	1	Uremia
9. C-55-F	Gastrointestinal malignancy; uremia	Stuporous	73	6.6	1	1.5	10	Chronic nephritis with uremia
10. C-54-F	Hypertension and hypertensive heart disease	Coma	126	19.0	1	2.0	3	Postmortem—Ca. of cervix with obstruction to the ureters with uremia
11. C-53-M	Carcinoma of prostate	Disoriented	–	–	1	3.0	4	Ca. of prostate. Arteriosclerosis with uremia

Low Xanthoprotein Reactions.—This group comprises 68 cases in which the xanthoprotein reaction was below the value of one, considered low, or negative. The results were not uniform; the diagnoses were varied, and the prognoses were uncertain.

Tables III and IV list the data of 24 patients who died, one group with a high urea nitrogen value and another group with low values. The xanthoprotein values ranged between 3 and 9. Two patients showed an indican value of 2.0 mg. per cent just below the uremic level.

TABLE III

LOW XANTHOPROTEIN REACTION—HIGH UREA NITROGEN, PATIENTS DIED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD				DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
1. C-64-M	Uremia in coma	Coma	100	9.5	9	0.15	5	Coronary thrombosis
2. C-105-M	Prostatic hypertrophy	Senile	55	4.0	8	2.0	23	Prostatic hypertrophy; alkaline cystitis
3. W-62-F	Hypertension Uremia	Semistuporous	48	2.8	9	0.05	1	Postmortem—Mod. ecc. hypertrophy of heart
4. C-38-F	Chr. morphinism Asthma		56	3.6	4	1.5	34	Postmortem—Genl. amyloidosis (chr. morphinism)
5. W-68-M	Syphilitic aortitis	Decompensated	3/7/34 33 | 3/27/34 43	2.1 | 2.6	3/15/34 8	–	16	Malignant endocarditis
6. W-33-M	Chr. glomerulonephritis; uremia	Coma	33	4.0	6	–	19	Postmortem—Chr. glomerulonephritis and bronchopneumonia
7. C-67-M	Hypertension with arteriosclerosis		11/6/33 115	10.0	11/4/33 4	1.0	23	Arteriosclerosis
8. C-32-F	Chr. glomerulonephritis		12/6/33 116	–	12/12/33 4	2.0	48	Postmortem—Chr. glomerulonephritis

Three of the cases were diagnosed as chronic glomerulonephritis at the postmortem examination. The blood chemistry and the xanthoprotein reactions were performed some time before death. A check on the xanthoprotein reaction at a later date would have been very desirable.

In two patients listed in Table III and in 9 patients listed in Table IV, the xanthoprotein reaction was performed shortly before death. The patients died of conditions other than renal involvements, and all had low xanthoprotein values. In the other cases too much time elapsed between the test and death to make conclusions of any value.

Table V lists 5 cases of patients, all with elevated blood urea nitrogen, who either left the hospital by discharge or release.

The 39 cases listed in Table VI present a wide range of diagnoses. The blood nitrogenous substances were not increased; the xanthoprotein reaction ranged from a value of 4 to 10, most of the values being in the group between 5 and 10. All the patients left the hospital improved.

Of interest is one case, Case 30, diagnosed as acute glomerulonephritis with a low xanthoprotein value of 8. This finding confirms Becher (1925) and other investigators who reported little or no increase of aromatic substances in acute nephritis.

TABLE IV

LOW XANTHOPROTEIN REACTION—LOW UREA NITROGEN, PATIENTS DIED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD				DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
1. W-59-M	Exposure; malnutrition	Acutely ill	21	–	7	–	13	Coronary sclerosis and hypostatic pneumonia
2. W-70-M	Arteriosclerosis		18	–	6	–	7	Arteriosclerosis with diabetic gangrene
3. C-68-M	Arteriosclerosis	Acutely ill	26	1.8	7	0.05	27	Arteriosclerotic heart disease
4. C-59-M	Pulmonary tuberculosis		10	–	9	–	5	Bronchiectasis and lung abscess
5. W-50-M	Malignant endocarditis	Acutely ill	28	2.0	5	–	1	Postmortem—Malignant endocarditis
6. W-68-M	Carbuncle, arteriosclerosis, diabetes		18	–	6	–	51	Carbuncle of shoulder and diabetes
7. W-63-M	Carcinoma of stomach and coronary sclerosis		23	1.7	7	–	13	Organic heart disease
8. W-67-M	Prostatic hypertrophy		37	2.3	6	–	4	Prostatic hypertrophy and organic heart disease
9. W-36-M	Malignant nephrosclerosis		32	2.0	7	0.05	13	Postmortem—Chr. glomerulonephritis
10. W-69-M	Malignancy or cirrhosis	Acutely ill	–	–	8	–	34	Carcinoma of stomach
11. W-57-M	Prostatic hypertrophy		16	–	3	0.05	2	Prostatic hypertrophy
12. C-30-M	Acute rheumatic arthritis		11	–	3	–	85	Postmortem—Psoas abscess, tuberculous caries lumbar vertebrae
13. C-49-M	Coronary disease	Decompensated	31	2.1	7	–	8	Senility
14. C-70-M	Aortitis	Decompensated	19	–	9	–	6	Hypertensive heart disease with aortitis. Bronchopneumonia
15. W-62-M	Carcinoma of thyroid	Acutely ill	20	–	7	–	4	Carcinoma of lung
16. W-54-M	Hypertensive heart disease	Congestive failure	15	–	8	–	1	Hypertensive heart disease

Routine Cases.—The blood from 57 routine cases of patients, with the exception of comas and nephritics, was obtained at the time of admission for study of the aromatic substances. All the xanthoprotein values were low, ranging from 4 to 10.

Postmortem Cases.—The xanthoprotein reaction was performed on the heart blood of 35 cases at postmortem. The test gave a value of 1 in 14 cases. The anatomic diagnosis revealed kidney involvement in 8 cases, liver pathol-

TABLE V

LOW XANTHOPROTEIN—HIGH UREA NITROGEN—PATIENTS DISCHARGED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD			DISPOSITION DAYS AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG. %	XANTH.		
1. W-47-M	Syphilitic heart disease	Acutely ill	36	2.3	6	Discharged 45 days	Coronary thrombosis Hemiplegia
2. W-54-M	Impending uremia	Convulsion	65	3.8	7	Discharged 15 days	Chronic glomerulonephritis
3. W-51-F	Generalized arteriosclerosis	Condition poor	131	9.5	3	Released 1 day	Uremia with chronic nephritis
4. W-61-M	Thyrotoxicosis		35	2.4	7	Released 7 days	Thyrotoxicosis
5. W-32-M	Nephrotic syndrome		75	5.4	7	Discharged 2 days	Chronic glomerulonephritis

ogy in 2 cases, and 1 case each of subacute bacterial endocarditis, cerebral hemorrhage, suppurative leptomeningitis, and carcinoma of the pancreas.

The xanthoprotein reactions obtained from the 2 cases of liver cirrhosis and the 1 case of carcinoma of the pancreas with liver metastases agree with the findings of other investigators. Tschilow (1929) reported high values in different types of liver diseases. Hoesch (1931) found elevated xanthoprotein values in cases of liver cirrhosis, acute liver atrophy, liver and bile duct carcinoma, and severe cholangitis. He believed that finding of a high xanthoprotein reaction, a relatively low nonprotein nitrogen and urea, along with a negative indican, could be used diagnostically to rule out renal disease, especially in comatose conditions.

The anatomic diagnosis showed renal pathology in 7 other cases, with xanthoprotein reactions ranging from 2 to 9.

The remaining 14 cases showed xanthoprotein values from 3 to 10.

Wuhrman (1935) studied the nonprotein nitrogen and the xanthoprotein reaction in living, agonal, and postmortem states and found an increase of these values in the agonal and postmortem states. This work casts doubt on the validity of results, such as the ones obtained in this series of postmortem cases.

Xanthoprotein Reaction Compared in the Blood and Body Fluids.—In 13 postmortem cases listed in Table VIII, the xanthoprotein reaction was performed on the heart blood, and on the pericardial fluid, on 2 cases of peritoneal fluid, and on two cases of pleural fluid. Chemical determinations were obtained on the pericardial fluid of 7 cases.

It is interesting to note that in 6 cases the same values for the xanthoprotein reaction were obtained in the blood and pericardial fluid, while in Case 5 the same value was obtained in the blood, peritoneal, and pericardial fluids. In some of the other cases there was a close agreement between the different values. Becher (1924) likewise found a corresponding increase in the xanthoprotein reaction of blood and body fluids.

TABLE VI

LOW XANTHOPROTEIN—LOW UREA NITROGEN—PATIENTS DISCHARGED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CONDITION	BLOOD			FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG. %	XANTH.	
1. W-25-M	Diabetic acidosis	Acutely ill	13		7	Acute appendicitis
2. W-50-M	Carcinoma of stomach		14		5	Carcinoma of stomach
3. W-64-M	Cystitis	Acutely ill	18		4	Acute cystitis
4. W-37-M	Exacerbation of chronic nephritis		20		7	Chronic nephritis
5. W-62-M	Leucemia		11		7	Chronic lymphatic leucemia
6. W-27-M	Toxic hepatitis. Neo-arsphenamine		12		8	Arsphenamine reaction
7. C-48-M	Arteriosclerotic heart disease		20		8	Syphilitic heart disease
8. W-51-M	Meningoencephalitis	Acutely ill	20		6	Organic heart disease Coronary disease
9. W-52-M	Chronic nephritis		18		9	Chronic nephritis
10. W-61-M	Sacroiliac arthritis		18		7	Sacroiliac arthritis
11. W-30-M	Functional neurosis		18		9	Chronic cholecystitis
12. W-29-M	Cholelithiasis		12		9	Gallbladder disease
13. W-38-M	Peptic ulcer		14		6	Chronic cholecystitis
14. C-39-M	Gonorrheal arthritis		13		6	Acute arthritis
15. W-75-M	Arteriosclerotic heart disease	Acutely ill	20		6	Coronary sclerosis
16. W-45-M	Lymphosarcoma		10		7	Hodgkin's disease
17. W-52-M	Hypertensive heart disease		16		6	Hypertensive heart disease
18. W-31-M	Rheumatic heart disease		15		5	Rheumatic heart disease
19. W-62-M	Carcinoma of stomach		9		5	Carcinoma of stomach
20. C-60-M	Infectious nonspecific arthritis		14		4	Infectious polyarthritis and generalized arteriosclerosis
21. C-57-M	Generalized arterio-sclerosis		25	1.8	7	Hypertensive heart disease
22. W-22-M	Exophthalmic goiter		12		9	Thyrotoxicosis
23. W-22-M	Hyperthyroidism		11		9	Thyrotoxicosis
24. W-53-M	Diabetes mellitus		10		6	Peptic ulcer and diabetes mellitus
25. W-33-M	Acute articular rheumatism		16		7	Rheumatic fever
26. C-48-F	Syphilitic aortitis		11		7	Syphilitic aortitis
27. W-46-M	Sciatica		10		5	Sciatica
28. W-69-M	Hypertensive heart disease		20		6	Hypertensive heart disease
29. W-41-F	Hypertensive heart disease		33	2.4	7	Hypertensive heart disease
30. W-36-M	Exacerbation Chr. glomerulonephritis		21		8	Acute glomerulonephritis
31. C-62-M	Syphilitic heart disease	Congestive failure	14		6	Syphilitic heart disease
32. W-79-M	Chr. myocarditis		17		9	Chr. myocarditis and arteriosclerosis
33. C-38-M	Hypertensive heart disease	Congestive failure	10		8	Hypertensive heart disease
34. W-53-M	Diabetes mellitus Hemiplegia		10		7	Diabetes mellitus. Hemiplegia
35. W-41-M	Acute articular rheumatism		13		7	Acute articular rheumatism
36. W-69-M	Arteriosclerotic heart disease		15		6	Arteriosclerotic heart disease
37. W-55-M	Arteriosclerotic diabetes		15		10	Coronary thrombosis
38. W-66-M	Dyspepsia		18		6	Nervous dyspepsia
39. W-48-M	Nontoxic adenoma of thyroid		9		5	Nontoxic adenoma of thyroid

TABLE VII

POSTMORTEM CASES

POST-MORTEM NO.	RACE AGE SEX	PREMORTEM BLOOD CHEMISTRY		POSTMORTEM BLOOD		HOURS DEAD	POSTMORTEM DIAGNOSIS AND COMMENTS
		UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
1. 1017	C-21-M			10	0.05	17	Ruptured aortic aneurysm
2. 1082	W-46-F			6	1.0	4	Chronic glomerulonephritis. Coma before death
3. 1122	C-66-F	23	1.7	7		?	Syphilitic aortitis
4. 1149	W-23-F			9	0.05	4	Ruptured aneurysm of a cerebral artery
5. 1155	C-20-F			3	0.1	1½	Generalized sepsis. Acute diffuse interstitial nephritis
6. 4	W-59-M			3	1.0	14	Carcinoma of prostate. Malignant endocarditis
7. 19	W-42-M	80	7.5	9	2.0		Stricture of urethra. Bilateral pyonephrosis and ascending suppurating pyelonephritis
8. 34	W-72-M	32	2.0	6	0.05	1	Carcinoma of prostate
9. 37	W-3½-F			6	0.05		Cystic supracellar adamantinoma
10. 64	C-43-F	23	1.7	7		4¾	Fibrocaseous pulmonary tuberculosis
11. 65	W-58-F			5			Sepsis lenta
12. 95	W-48-M	168		1	1.5	7½	Malignant nephrosclerosis
13. 244	W-50-M	102	7.0	1	1.5	5½	Chronic glomerulonephritis
14. 317	W-47-M	41	2.6	4	0.35	35	Subacute to chronic glomerulonephritis
15. 386	W-29-M			1	0.4		Subacute vegetative endocarditis
16. 413	W-63-M	66	4.0	1	2.5	1½	Renal decompensation of benign nephrosclerosis and confluent bronchopneumonia
17. 428	W-57-M	9		1		2½	Periportal cirrhosis of liver
18. 492	C-40-F	100	9.3	1	2.5	19	Fibrinous pericarditis and cyst of left ovary with compression of the uterus and left ureter
19. 496	W-48-F	15		5	0.05	2	Hydropericardium. Hypertrophy of left ventricle of heart. Arteriosclerotic granulations of kidneys
20. 500	W-38-M			1		2½	Cerebral hemorrhage
21. 508	C-38-M	62	4.4	6		15½	Ancient myomalacia of apex of heart
22. 516	W-54-M			1		5½	Suppurative leptomeningitis (otitic)
23. 528	C-54-F	126	19.0	1 Premortem blood: Xan. 1 Ind. 2 mg.			Ulcerated carcinoma of the cervix and compression of the ureteral openings
24. 530	C-38-F	44	2.5	1	0.5	16	Malignant nephrosclerosis. Xanth. 5-6 days before death
25. 548	W-86-M			4		1¾	Recent encephalomalacia of left side of pons
26. 552	C-50-F			1		2	Carcinoma of the pancreas
27. 559	W-39-M	12		1		9	Periportal cirrhosis of the liver
28. 569	C-54-F			5	1.5		Ulcerative esophagitis with perforation and suppurative lymphadenitis of posterior mediastinum

TABLE VII—CONT'D

POST-MORTEM NO.	RACE AGE SEX	PREMORTEM BLOOD CHEMISTRY		POSTMORTEM BLOOD		HOURS DEAD	POSTMORTEM DIAGNOSIS AND COMMENTS
		UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
29. 566	W-60-M	34	2.2	4		6	Marked eccentric hypertrophy of heart
30. 594	C-37-M	144	11.0	1	2.5	16½	Malignant nephrosclerosis
31. 599	C- 5-M			3	2.0	19	Arsenic poisoning
32. 598	C- ?-M			4	2.5	12	Heat prostration (clinically)
33. 600	W-67-M	18		2	0.3	2	Carcinoma of prostate Bilateral ascending pyelonephritis
34. 606	C-46-F	9	140.0	1	0.4	18	Chronic glomerulonephritis
35. 632	C-63-M	33	2.2	7	0.6	1½	Glandular hypertrophy of prostate gland

TABLE VIII

BODY FLUIDS

RACE AGE SEX	ANATOMIC DIAGNOSIS	BLOOD		PERICARDIAL FLUID						HOURS DEAD
		XANTH.	INDICAN MG. %	UREA N. MG. %	CREATININE MG. %	SUGAR MG. %	CHLORIDES MG. %	XANTH.	INDICAN. MG. %	
1. C-40-F	Fibrinous pericarditis	1	2.5	172	9.6	-	-	1	2.5	19
2. W-48-F	Hydropericardium Hypert. l. ventricle of heart	5	0.05	23		133	-	7	0.05	2
3. C-38-M	Ancient myomalacia	6						5		15
4. W-54-M	Suppurative leptomeningitis	1						4		5½
5. C-54-F	Carcinoma of cervix	1	Peritoneal fluid, Xanth. 1					1		15
6. C-38-F	Malignant nephrosclerosis	1	Pleural fluid, Xanth. 4					1		16
7. W-86-M	Encephalomalacia of pons	4		31	2.3	96	764	6		1¾
8. C-50-F	Carcinoma of pancreas	1	Pleural fluid, Xanth. 4					3		2
9. W-39-M	Periportal cirrhosis of liver	1	Peritoneal fluid, Xanth. 2					1		9
10. W-60-M	Eccentric hypertrophy heart	4		84	5.0	30	660	4		6
11. C-37-M	Malig. nephrosclerosis	1	2.5	233	15.7	78	560	1	0.45	16½
12. W-67-M	Carcinoma of prostate gland	2	0.3	71	5.0	80	735	4	0.4	2
13. C-46-F	Chronic glomerulonephritis	1	0.35	168	10.0	36	585	1	1.0	18

SUMMARY AND CONCLUSIONS

Evidence is presented in this paper to confirm the findings of Becher and other investigators who reported very high value of the xanthoprotein reaction in cases of marked renal insufficiency either in uremia or approaching

uremia. All the cases in this series with a xanthoprotein reaction of one or stronger were diagnosed as cases of renal insufficiency, and died. For practical purposes any xanthoprotein reaction of a dark yellow, golden color may be considered positive or equivalent to the values expressed as one in this work. According to the work of Rose and Alving the xanthoprotein reaction with a value of one is equivalent to a tyrosine content of the blood of 7.2 mg. per cent and a kidney function of 10 per cent of normal. Many of the xanthoprotein reactions with a positive value are more intense than the 0.03874 per cent potassium dichromate solution. Hence, a phenol concentration equivalent to a tyrosine concentration of 7.2 mg. per cent or higher must be considered when evaluating the xanthoprotein reaction of one.

The author concludes that the real significance of the xanthoprotein reaction is in the information obtained from a positive value of one or stronger.

Xanthoprotein reactions of less intensity than one were found hard to evaluate. Values from 2 to 5 were found in a great variety of patients, some of whom lived, and some of whom died. However, in general, the patients with values between 5 and 10 improved, lived, and were discharged.

As has been pointed out by other investigators, no correlation was found between the aromatic and nitrogenous products in the blood.

The findings—that, in general, the indican and the xanthoprotein reactions parallel each other, but in some cases the indican may be low and the xanthoprotein high—confirm the results obtained by Becher.

The author concludes that the xanthoprotein reaction is more sensitive than the indican test in the diagnosis and prognosis of renal insufficiency.

The xanthoprotein reaction of one proved to be 78.5 per cent correct in the diagnosis of marked renal insufficiency and liver disease in the postmortem cases.

A general correlation was observed between the xanthoprotein reaction in the blood, the pleural, pericardial, and peritoneal fluids, indicating that a similar increase of aromatic substances was found in the body fluids when they were augmented in the blood.

The xanthoprotein reaction commends itself in that it is easily, rapidly, and accurately performed with a definite aid to diagnosis and prognosis.

REFERENCES

1. Becher, E.: Über das Vorkommen aromatischer Gruppen in entweisstem Blut, Körperflüssigheiten und Geweben, nachgewiesen am Ausfall der Xanthoproteinreaktion, München. med. Wchnschr. **71**: 1677, 1924.
2. Becher, E.: Über eine neue einfache Methode zu Feststellung der Niereninsuffizienz im Blut, München. med. Wchnschr. **71**: 1611, 1924.
3. Becher, E.: Studien über das Verhalten der Xanthoproteinreaktion im enteiweissten Blut unter normalen und pathologischen Verhältnissen, Deutsche Arch. f. Klin. Med. **148**: 159, 1925.
4. Becher, E.: Über Unterschiede im Verhalten des Blutes bei Niereninsuffizienz der akuten Nephritis und der Schrumpfnieren und die Verwertbarkeit der Xanthoproteinprobe im einweissfreien Blutfiltrat für die Diagnose und Prognose der Niereninsuffizienz, München. med. Wchnschr. **72**: 1020, 1925.
5. Becher, E., and Koch, F.: Über die pathogenetischen Beziehungen zwischen echter Urämie und den bei Niereninsuffizienz im Blut retinierten Substanzen, Deutsche Arch. f. Klin. Med. **148**: 78, 1925.
6. Becher, E., Litzner, S., and Taglich, W.: Über das Vorkommen von Phenolen im normalen Blut, über ihren qualitativen und quantitativen Nachweis mit der Millonschen Reaktion und über bemerkenswerte Blutphenolwerte bei Krankheiten inbesondere bei perniziöser Anaemie, München. med. Wchnschr. **72**: 1676, 1925.

7. Becher, E., Litzner, S., and Taglich, W.: Der Phenolgehalt des Blutes unter normalen und pathologischen Verhältnissen, Ztschr. f. Klin. Med. **104**: 182, 1926.
8. Becher, E.: Welche diagnostischen Schlüsse lassen sich aus starken Verschiedenheiten im Ausfall der Indikan- und Xanthoproteinprobe ziehen? Eine neue Einteilung der Urämia, München. med. Wchnschr. **77**: 432, 1930.
9. Boeminghaus: Über den Wert der Xanthoproteinreaktion für die Beurteilung der Nierenschädigung bei chronischer Harnstauung, Ztschr. f. Urol. **20**: 881, 1926.
10. Goldman, M., and Burniewicz, J.: Über den klinischen Wert der Xanthoproteinreaktion im Blut, Ztschr. f. Klin. Med. **107**: 716, 1928.
11. Gromer, V.: Zur Anwendung der quantitativen Bestimmung des Xanthoproteinwertes des Blutserum nach Becher als Methode für den Nachweis der Niereninsuffizienz, Zentralbl. f. Inn. Med. **472**: 1125, 1926.
12. Hoesch, K.: Chemische Blutwerte bei echtem Koma und komaähnlichen Zuständen Leberkranker, Ztschr. f. Klin. Med. **117**: 175, 1931.
13. Inoue, H.: Klinische und experimentelle Untersuchungen über Xanthoproteinreaktion des Blutes bei Leberschädigungen, J. Chosen M. A. **23**: 94, 1933.
14. Kammer, L.: Über die Xanthoproteinreaktion im Blutserum, Ztschr. f. Klin. Med. **125**: 632, 1933.
15. Melly, Bela: Über den Wert Xanthoproteinreaktion im enteiweissten Blut in der urologischen Chirurgie, Ztschr. f. Urol. Chir. **24**: 581, 1928.
16. Monias, B., and Shapiro: The Value of the Indican Determination in the Blood in Cases of Renal Insufficiency, Arch. Int. Med. **45**: 573, 1930.
17. Peters and Van Slyke: Quantitative Clinical Chemistry, Baltimore, 1931.
18. Rakestraw, N. W.: A Quantitative Method for the Determination of Phenols in Blood, J. Biol. Chem. **56**: 109, 1923.
19. Rakestraw, N. W.: Chemical Factors in Fatigue. II. Further Changes in Some of the Blood Constituents Following Strenuous Muscular Exercise, J. Biol. Chem. **56**: 121, 1923.
20. Rathery, F., and Waitz, R.: Réaction Xanthoproteique dans les Affections Renales, Compt. rend. Soc. de Biol. **103**: 214, 1930.
21. Rose and Alving: Personal Communication.
22. Scherk, G.: Über die klinische Verwertbarkeit der Xanthoproteinreaktion im Serum bei Nierenkrankheiten, Med. Klin. **23**: 133, 1927.
23. Smith, M. I.: The Estimation of Tissue Phenols, Pub. Health Reports **48**: 1487, 1933.
24. Theis, R. C., and Benedict, S. R.: Phenol and Phenol Derivatives in Human Blood in Some Pathological Conditions, J. Biol. Chem. **36**: 99, 1918.
25. Tonietti: Importanza della determinazione della sostanze aromatiche de sangue nelle malattie renali, Policlinico (sez. med.) **36**: 163, 1929.
26. Tschilow, K.: Über den klinischen Wert der Xanthoproteinreaktion (X. P. R.) im Blute, Wien. Arch. f. inn. Med. **18, 19**: 67, 1929-30.
27. Widenhorn, H.: Die Xanthoproteinreaktion, die Urochromogenreaktion und die Kaolinprobe als Nierenfunktionsprüfungen, Ztschr. f. Urol. Chir. **25**: 215, 1928.
28. Wuhrman, F.: Reststickstoff und Xanthoproteinreaktion im Agonal- und Leichenblut, Ztschr. f. Klin. Med. **127**: 499, 1935.
29. Zamyslowa, K.: Die Bestimmung des Indicans und der Xanthoproteinreaktion im Blute als Methode der Nierenfunktionsprüfung, Ztschr. f. Klin. Med. **116**: 732, 1931.

AN INEXPENSIVE FORBUS TYPE AUTOPSY TABLE*

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RECENTLY it became necessary to replace an outmoded autopsy table at this institution. Because of the exorbitant prices asked by supply houses for tables offering any conveniences beyond those inherent in a drainable stationary slate or metal slab, recourse was had to the literature, with the thought of constructing a table from published plans. After some deliberation, the table designed by Forbus[1] was taken as a model, and using salvaged materials for the most part, a table was built by the maintenance department of this institution.

The table, which has now been in use for several months, has been more than satisfactory. In view of its low cost without sacrifice of convenience, we thought it worth while to publish complete plans and specifications of our model. Our plans present no great departures from the general design of the original, but do provide for many substitutions of material by which sizable economies were effected without loss of utility. The changes we have made may be appreciated by comparing our specifications with the specifications contained in Forbus' article. Labor and material costs are given as of 1936. Although practically all of the materials which we utilized were salvaged, costs given here are for new materials. Plans and a photograph are included which convey an adequate impression both of the details of construction and the finished appearance.

SPECIFICATIONS

Table Top.—The top consists of seven pieces of light gray marble, 7/8 of an inch thick, set with waterproof cement in an angle iron frame 32 1/8 by 93 3/4 inches constructed of 2 by 2 angles 1/4 of an inch thick. Angle members support all joints throughout their entire lengths, thus making the table top watertight. The working surface is highly polished and is graded one inch from the head of the table to a drain at the foot. The two pieces forming the main part of the table top are fitted tightly inside the end and side curbs which locks the curbs between the main part of the table and the angle iron frame as shown in Sections AA and BB. A shelf 15 inches wide extending across the width of the table is sloped 1/4 of an inch from the foot to the drain. The edge of the shelf at the drain is bull nosed (see Section AA).

Twenty-six inches from the inside of the head and on the left side of the table, an opening 8 by 12 inches is cut for the sink. The outer margin of the opening is formed by the side curb and angle iron frame (see Section CC).

At the head of the table two holes are bored for 1/2 inch water inlet pipes and at the foot of the table on the center line a hole is bored for a 1 1/2 inch

*From the Department of Pathology and Department of Maintenance of the Pittsburgh City Home and Hospitals.
Received for publication, June 5, 1936.

waste line. The waste is fitted with a 3-inch brass strainer countersunk flush with the surface of the table (see the general plan and Section AA).

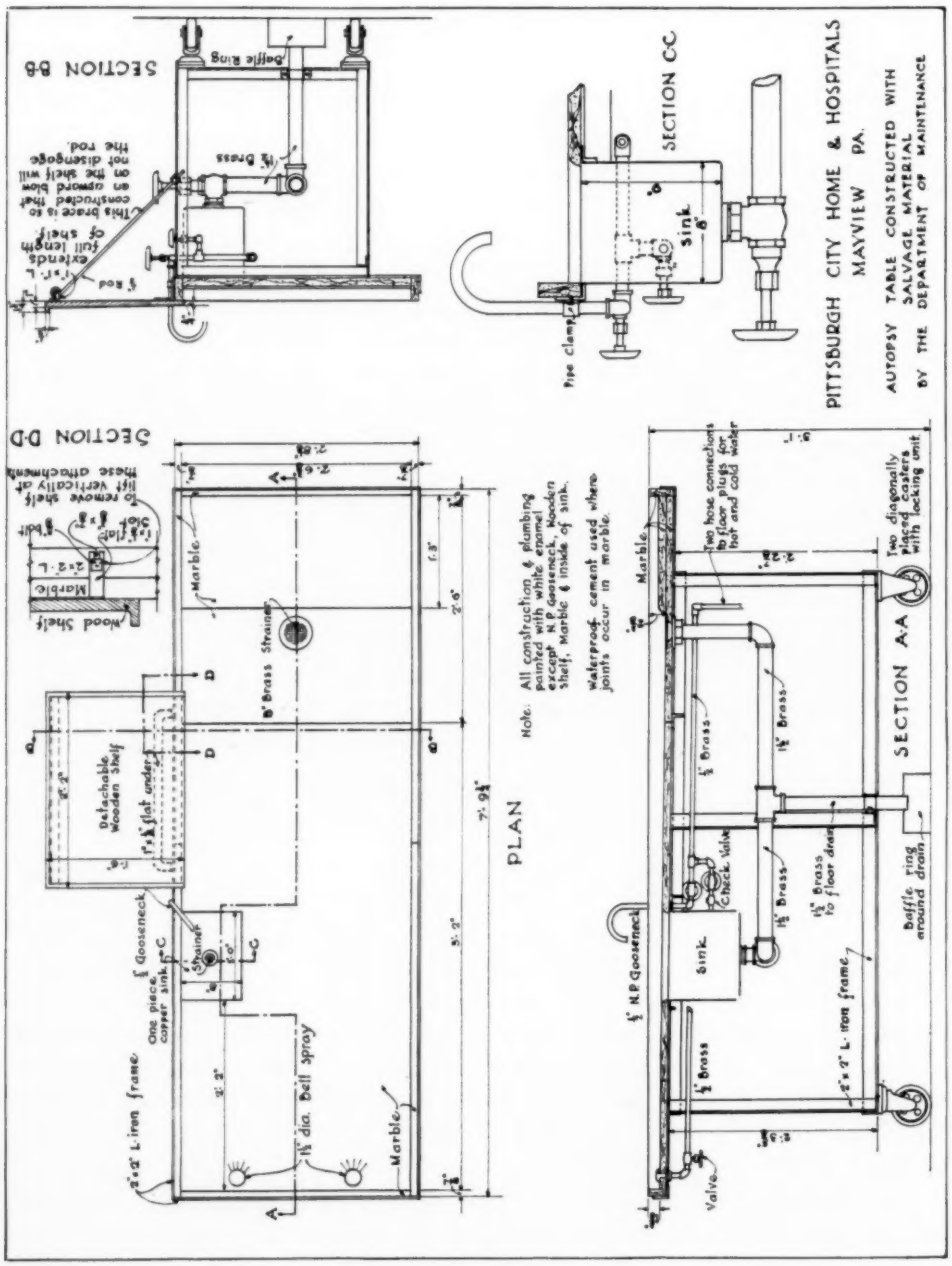
PITTSBURGH CITY HOME & HOSPITALS
MAYVIEW PA.
AUTOPSY TABLE CONSTRUCTED WITH SALVAGE MATERIAL BY THE DEPARTMENT OF MAINTENANCE
SECTION B-B
SECTION C-C
SECTION D-D
SECTION A-A
PLAN
Baffle Ring
Marble
Wood Shelf
Detachable Wooden Shelf
Strainer
Gooseneck
Sink
Valve
Check Valve
Baffle ring around drain
Two diagonally placed casters with locking unit
Two hose connections to floor plugs for hot and cold water
Note. All construction & plumbing painted with white enamel except N.P. gooseneck, wooden shelf, Marble & inside of sink.
Waterproof cement used where joints occur in marble.

Fig. 1.

Table Base and Frame.—The table is supported by an angle iron frame constructed of 2 by 2 angle iron ¼ inch thick. The top frame consists of three longitudinal and six cross members, and is connected to the bottom frame by five vertical legs. One leg is placed on the left side of the table under the detachable

shelf, and also serves as a support for the angle rod of the shelf. The bottom frame consists of two longitudinal and three cross members. The middle cross member also supports the main waste line. Four rubber-tired casters are attached to the lower frame directly under the corner legs.

All joints are bolted and the bolt heads in contact with the marble are countersunk. The bolts in the top frame were left loose until the marble top was set and then tightened. This action assures tight joints and locks the marble firmly in place. All iron work, plumbing, and the under side of the marble top were painted with white enamel. (For measurements and details see the general plan and sections.)

Plumbing.—Two 1½ inch brass drain pipes, one from the small sink and one from the screen at the foot of the table join at the center of the table and

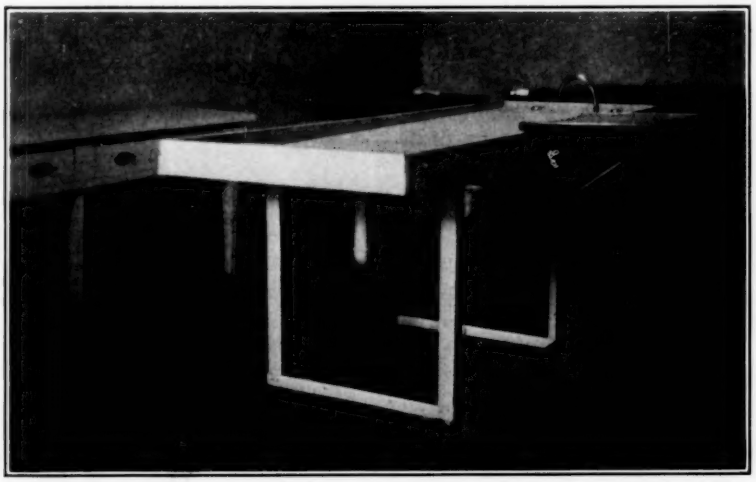

Fig. 2.—Photograph of completed autopsy table.

discharge over a floor drain equipped with a removable copper baffle ring to prevent splash. The drain from the sink is controlled with a 1½ inch valve (see Sections AA, BB, and CC).

The water supply lines are ½ inch red copper pipe except the nickel-plated gooseneck supply to the sink. The main supply begins at the foot of the table and extends along the center line to the head of the table. At the foot of the table the main line has two branches which are connected with rubber hose to hot and cold water supplies which are valved at the floor. A single branch off the main line supplies both inlets to the sink and each inlet is valved separately. The side inlet to the sink is equipped with a check valve to prevent syphoning. The main line terminates at the head of the table in two 1½ inch bell inlets with fan-shaped apertures which spray water over the surface of the table. All pipes are secured to the under side of the top angle frame with clamps and bolts. All valves are turned to the outside of the table for ease of operation (see Sections AA, and BB).

The sink is made of sixteen gauge copper, bright on the inside. The outside is painted white. Its dimensions are 8 inches wide, 12 inches long, and 9 inches deep. A flange ¾ of an inch wide around the top perimeter is locked between the bottom of the marble top and angle iron frame to hold the sink rigidly in place (see Sections AA, and CC).

Detachable Wooden Shelf.—The shelf is made of white pine painted with white enamel on the under side and orange shellac on the top. The shelf slopes slightly from the outer edge to the inside edge which projects ¼ of an inch beyond the curb. Although the shelf is easily removed, its attachments are so constructed that it will not tilt and cannot be accidentally disengaged (see Sections BB, and DD).

Cost of Materials—1936:	
Marble for top	$ 28.00
Angle iron	5.60
Pipe valves and fittings	19.00
Copper sink	1.40
Casters, rubber-tired	12.00
Paint	4.00
Total material	$ 70.00
Cost of Labor—1936:	85.00
Total cost	$155.00

REFERENCE

1. Forbus, W. D.: An Autopsy Table: A New Design, Arch. Path. **14**: 506, 1932.

FRACTIONAL TRANSFUSION*

WARD J. MAC NEAL, M.D., AND MARGARET E. (STRAUB) NEIL, NEW YORK, N. Y.

THE transfusion of a large quantity of compatible blood is ordinarily a Bland procedure when the recipient has just suffered a profuse hemorrhage and is otherwise in good condition. When, however, there is an extensive pneumonia, myocardial deficiency, septic thrombosis, or endocardial vegetations, the sudden introduction of a large volume of blood or other fluid into the vascular system may be followed by unfavorable or even disastrous results. We have found that these difficulties may be avoided by using citrated blood and injecting small amounts at intervals during the day, a method which we designate as fractional transfusion.

The blood from the donor is received into a suitable bottle of about 350 c.c. capacity. This bottle is fitted with a rubber stopper perforated by two glass tubes. One of these is bent at a right angle and its tip† is ground to fit a standard Luer needle. The lumen at the tip is drilled to a diameter of at least 1.5 mm.

*From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

Received for publication, July 7, 1936.

Aided by a grant from the Josiah Macy, Jr., Foundation.

†The ground glass tips to fit Luer needles can be obtained from Eimer and Amend, Third Ave. and 18th Street, New York City, and probably from many other dealers in glass apparatus.

so as to permit the free flow of blood. The lower end of this angle tube projects into the bottle for about 20 mm. below the rubber stopper. The second hole in the rubber stopper accommodates a straight glass tube plugged with cotton. Its lower end extends only to the level of the lower end of the rubber stopper. After covering the angle tube with a small test tube the entire apparatus is sterilized by steam under pressure. After sterilization one places 30 to 50 c.c.

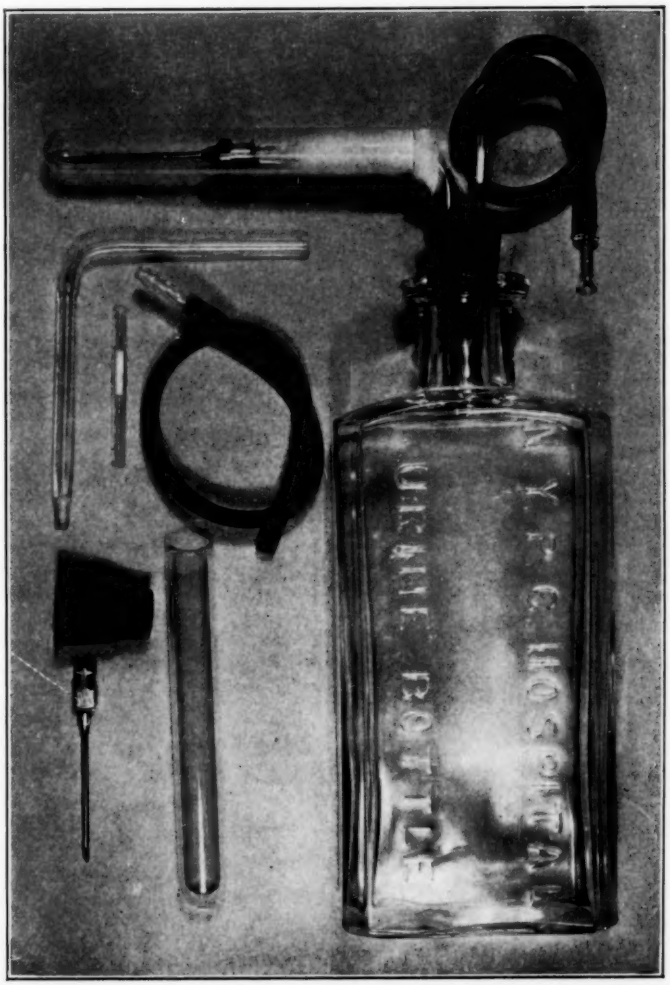

Fig. 1.—Bleeding bottle assembled ready for addition of the citrated saline solution. The separate small parts are shown at the left: (*1*) Curved inlet tube with tip ground to fit the needle, (*2*) straight outlet tube plugged with cotton, (*3*) rubber suction tube with glass mouthpiece, (*4*) rubber stopper with two holes, (*5*) needle, gauge 15, and (*6*) protective test tube.

of sterile 5 per cent sodium citrate in 0.9 per cent saline in the bottle, best by means of a Pasteur bulb pipette. A small firm rubber suction tube is attached to the straight outlet tube and a No. 15 gauge needle, sterilized by boiling in oil, is fitted to the ground end of the angle inlet tube.

The donor is placed in a recumbent position and a garter tourniquet[1] is applied to his upper arm so as to cause maximum distention of the veins of the

forearm. The skin is prepared with iodine and alcohol. Then the needle is passed into one of the large veins at the elbow, preferably with its point directed toward the wrist. The blood should flow in a stream into the bottle so as to fill it in two to four minutes. Suction hastens the flow, and this is also favored by steady muscular movement of the patient, closing and opening his fist. After filling the bottle, the donor's arm is dressed and, after a brief period of observation, he is dismissed to duty.

The citrated blood in the bottle is immediately mixed, best by drawing part of it up into a large bulb pipette and discharging this again into the original bottle. One then transfers the blood to large sterile tubes, each containing about 50 c.c., and these are stored in the refrigerator ready for use. Such blood keeps for several days, but we have not had occasion to use it after the fourth day. The corpuscles tend to become gummy so that the older blood does not work so well in the injection syringe. It is unnecessary to warm the blood before injecting it into the patient.

For injection into the patient, we use syringes of resistance glass, capacity 50 c.c., sterilized by steam under pressure. Large Pyrex glass tubes are used to protect the syringe, one for the barrel of the syringe and a slightly narrower one for the plunger, such that these two tubes will telescope later to hold the filled syringe. One attaches a No. 15 needle to the syringe, draws in 15 to 20 c.c. of sterile saline and withdraws the plunger to the end of the barrel so as to spread a complete film of saline solution between the barrel and plunger. The saline solution is left in the syringe to mix with the blood, 40 to 45 c.c., which is drawn in rapidly through the large needle. This needle is then replaced with a smaller one, ordinarily gauge 24, for injection into the patient's vein. An operator with powerful hands may be able to use even a 27 gauge needle but this usually offers so much resistance to passage of the blood that it is not recommended. Some operators will prefer a 22 gauge needle. The smaller needle does less damage to the patient's vein.

Ordinarily we insert the needle directly into the prepared arm of the patient and into the properly distended vein without difficulty. Penetration of the vein can usually be recognized by the experienced operator through the sense of touch. If the injection is started while the needle is outside the venous lumen, this error becomes manifest by increased resistance and the formation of a small rounded swelling (hematoma) under the palpating finger, a mishap which requires immediate withdrawal and application of moderate pressure. The beginner, and also sometimes the experienced operator in a difficult case, will prefer to puncture the patient's vein with a suitable needle (gauge 24) attached to a small syringe of saline solution and to assure himself of successful penetration of the vein by withdrawal of a little blood into the syringe or, when the patient's blood pressure is so low that this fails, by injecting the saline solution into the vein. When the position of the needle is satisfactory, it is then grasped firmly by forceps and held in position while the small syringe is replaced by the large one containing the blood. This procedure ordinarily requires the cooperation of an intelligent assistant but can, if necessary, be done by one person working alone. The blood is injected in a period of two to five minutes. If

desired, further doses may be introduced at once through the same needle after exchange of syringes. However, this is ordinarily undesirable, for the chief advantage of the method is due to spacing out of the doses. Upon withdrawal of the needle, pressure is applied immediately to the puncture, and while the arm remains extended, the pressure pad is held in place by a garter tourniquet lightly applied for a full five minutes. The same vein may be used again after an hour or so. By using the arms alternately one may give the desired amount of blood in several injections at intervals of thirty minutes or more, as convenient, without risk of too sudden dilatation of the vascular system of the patient and without danger of other unexplained reactions. Even the rare allergic patient who may react badly to the most precisely matched blood will take the small doses at intervals without disaster; and by employing even smaller doses, administered still more slowly, even the mildest reactions may be avoided.

The disadvantages of the method relate particularly to the time-consuming labor involved in the cleansing and meticulous sterilization of the glassware and needles and the necessity for the operator to be available to inject the blood many times during the day. The advantages relate especially to the welfare of the patient who escapes all dangers of ill effects due to the rapid dilatation of his vascular system and is also spared the psychic shock of the first transfusion by the usual methods. To many very sick patients the repeated intravenous injections, performed at the bedside, are of little more significance than hypodermic medication. The method is also highly elastic in various respects. One may separate the blood into constituent layers and may at will give the patient blood plasma or the sedimented corpuscles suspended in saline solution. Such separation may be particularly desirable when one is employing immunized donors.

In our hands, the method has been especially valuable in the supportive treatment of patients suffering from severe sepsis and particularly in cardiac and pulmonary diseases in which the immediate behavior following larger transfusions has sometimes been disappointing. Daily multiple intravenous injections by this technic have been continued for months without serious injury to the veins.

REFERENCE

1. Straub, Margaret E., and Mac Neal, Herbert P.: A Tourniquet for Intravenous Procedures, Arch. Dermat. & Syph. **29**: 717, 1934.

A NEW AND SIMPLIFIED BLOOD CULTURE TECHNIC*

J. M. Feder, M.D., Anderson, S. C.

I HAVE struggled with every known method devised for obtaining a culture of the blood for the past twenty years and the results have been as variable as the means. Some were simple, some relatively efficient, some cumbersome, and others only a question mark, but all shared the same fault—undue handling of blood and media with consequent exposure to contamination. In the method described this main objection has been eliminated, while other desirable features have been added.

The method is simple. Kracke's media are employed for all routine work. The container is simply a six-sided, four ounce Pyrex nursing bottle, and its closure is the cap intended for that purpose, the Davol soft rubber nursing bottle cap.

We prepare our media in the usual manner as recommended by Kracke; 5 gm. of dry media, obtained from the Digestive Ferments Company of Detroit, is introduced into a container with 50 c.c. of distilled water. The flask is loosely plugged with gauze, and the cap is fastened to the neck with a rubber band. These are now sterilized at 15 pounds' pressure for twenty minutes. They are allowed to cool in the autoclave. Then with sterile-gloved hands the plugs are removed and discarded, and the tight-fitting rubber caps are stretched into place.† A large amount can be made at one time, as this media keeps indefinitely.

We keep a supply of 10 c.c. sterile syringes in large test tubes, the needles being protected from dulling on the bottom of the container by being encased in a section of glass rod slightly longer than the needle. This syringe together with the loaded, rubber-capped tube completes the armamentarium of the technician en route to the ward to obtain a blood culture.

The patient's arm is prepared in the usual manner. Ten cubic centimeters of blood is withdrawn and the top of the rubber cap having been either scrubbed with alcohol or painted with tincture of iodine is pierced with the needle and the blood expelled into the media and well shaken. No preliminaries are engaged in. Incubation is started at once. When subcultures are desired or examinations are to be made to determine presence of growth, material for the purpose is obtained in the same manner as when inoculated; through the cap. Two cubic centimeter hypodermic syringes are kept on hand for this purpose, sterilized in small test tubes, and the needle is simply thrust through the freshly asepticized cap and the desired amount withdrawn.

*From the Department of Pathology, Anderson County Hospital.

Received for publication, July 16, 1936.

†We have found that the rubber caps will go into place more easily if they are filled with water before sterilization. This water-filled cap is simply pressed into place.

In order to secure the required partial vacuum, we have found it necessary to open the sterilizer while still hot and immediately close the tubes. The required vacuum will be noted by the dimpling inward of the rubber cap.

In addition to the elimination of contamination, this procedure possesses the virtues of economy and easy portability. It may be carried in the bag in any position, for this flask is not harmed by being carried on its side or inverted. This is in sharp distinction to the cotton-stoppered containers where any deviation from the upright spells disaster. This is quite an advantage to those who, like me, may be frequently called upon to take blood cultures at the bedside away from the hospital. Another readily apparent advantage is that men remote from immediate laboratory facilities can obtain cultures in this container, as no great technical skill is required, and mail the container to the laboratory.

It has many advantages over its nearest competitor, the media-laden vacuum tube with needle attached. It is far less fragile. The cost is far less and the difficulty of obtaining blood through the broken, constricted neck of the vacuum

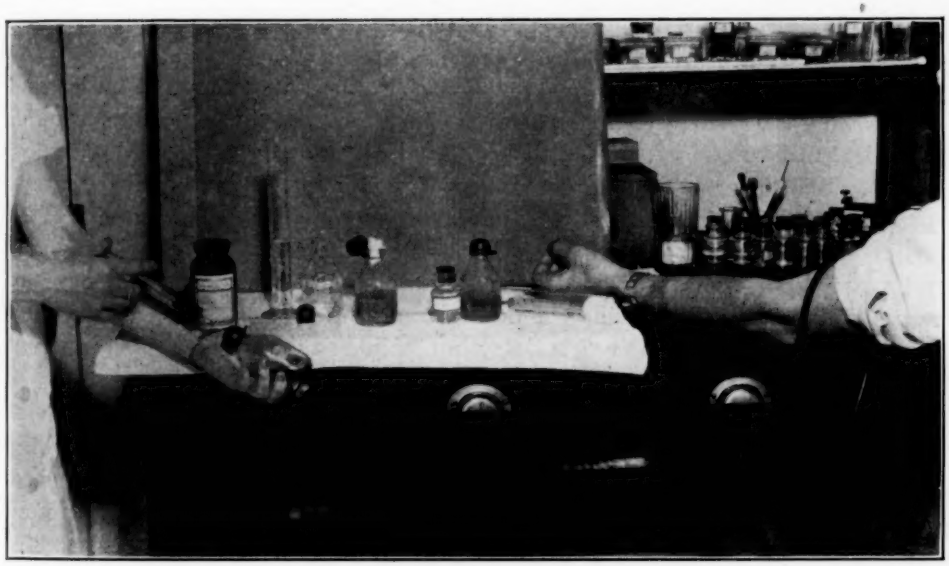

Fig. 1.

tube is eliminated. Not to be overlooked, also, is the frequently cut finger from the fragments of glass at the point of fracture of the glass neck.

SUMMARY

1. A simple, economical, practical and "fool-proof" method designed to facilitate the making of blood cultures is described.

2. A rubber cap is utilized and the blood culture is inoculated and withdrawn in the same manner in which vaccines and serums are obtained from their containers, i.e., *through* the rubber cap.

3. Being easily portable and safely carried in any position, the container may be carried about in a bag and mailed in when required.

4. As no special technical skill is required, our method places blood cultures at the disposal of the physician regardless of how remote his location may be.

CONTINUOUS REGISTRATION OF RECTAL TEMPERATURE DURING TREATMENTS IN THE HYPERTHERM*

WILHELM STENSTROM, PH.D., IRWIN VIGNESS, PH.D., AND CARL E. NURNBERGER, PH.D., MINNEAPOLIS, MINN.

THE inducement of artificial fever or hyperpyrexia has become a widely used method of treatment for patients suffering from general paresis, gonorrheal arthritis, and a few other diseases. The five methods commonly used consist in: (1) wrapping the patient in blankets and waterproof material such as rubber sheets, (2) wrapping the patient as in the first method but speeding up the process by means of diathermy treatments, (3) using short-wave diathermy, (4) enclosing the patient in a box and supplying heat by means of light bulbs, and (5) placing the patient in a large box in which moist air is heated by means of electrical resistances and circulated by a fan as is done in the Kettering hypertherm. Whichever method is used, it is of utmost importance to watch the temperature of the patient carefully. In most instances this is done by means of an ordinary rectal thermometer which has to be inserted and removed at short intervals. This is a very inconvenient method. A considerable amount of warm moist air escapes during the process and a fluctuation in the temperature may thus be produced. A certain delay in obtaining the temperature is inherent in this method and that may occasionally lead to complications.

By means of electrical devices for measuring temperatures, it is possible to read the temperature at any moment without delay and without disturbing the patient. Such devices are not suitable when short-wave diathermy is used, but for all the other methods of inducing heat it is practical. We have found the thermocouple method very satisfactory, but a resistance type thermometer may be used instead. A suitable apparatus can easily be assembled after the few necessary instruments have been obtained. The initial expense and effort are well rewarded by the simple and satisfactory manner in which the temperature fluctuations can be followed. A physician in charge of treatments of this kind is unwilling to give up this method after once it has been tried. By means of a few simple additions and special arrangements it is possible to obtain continuous photographic registration of the temperature. The complete self-recording apparatus, which has been constructed here, will be described. The simplification which can be made if the photographic recording is unnecessary is self-evident.

A copper—constantan (or advance)—thermocouple is used. One junction is kept at constant temperature in a thermostat constructed in accordance with the description of Harry Clark.† The other junction is usually placed in a

*From the Section of Biophysics and Cancer Institute, University of Minnesota, Medical School.

Received for publication, July 18, 1936.

†J. Exper. Med. 35: 385, 1922.

specially constructed rectal applicator. The temperature difference is registered by means of a d'Arsonval galvanometer. One-half of the light beam reflected by the galvanometer mirror is directed toward a transparent scale and the other half strikes a photographic paper mounted on a rotating drum. The details of the apparatus can be visualized by the cross-section in Fig. 1 to which the following description refers.

A wooden box B_1 100 cm. long, 35 cm. wide, and 44 cm. high was made to hold the instruments. The top and the long sides are removable. The bottom extends 23 cm. beyond one end. A smaller box B_2 holding the rotating drum D can be attached to this extension and wall W_1. The galvanometer G, a low resistance Leeds and Northrup type P, with a sensitivity 1.5 microvolts per mm. at 1 meter, is mounted on the wall W_2. The telescopic light projector L with a ½ amp. 4 volt line-filament light bulb and focusing lens is placed in a suitable position and can be adjusted so that the light after being reflected by the galvanometer mirror is focused on the photographic paper. A mirror M is placed so that one-half of the light beam is reflected and focused on a translucent scale

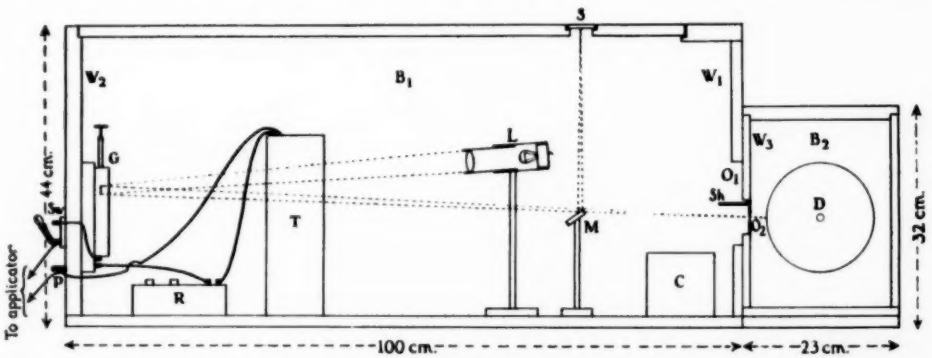

Fig. 1.—Apparatus for indicating and recording temperatures measured by a thermocouple applicator.

S placed on a narrow glass window in the cover. It is convenient to arrange this scale so that it can be moved laterally for zero adjustment. By means of this arrangement a reading can be taken at any time while the photographic registration is made. Openings O_1 and O_2 have been cut at the correct height in the adjoining walls W_1 and W_3 to let the light beam pass through. Box B_2 is supplied with a shutter, Sh, which can be pushed in front of slot O_2 when no exposure of the paper is desired and the box can thus be made light tight and can conveniently be carried to the dark room for exchange of photographic paper. The drum D is rotated once in twenty-four hours by means of a clockwork which is mounted on the outside of one of the walls of B_2.

A step-down transformer C supplies current for the light bulb. A small light bulb attached to the outside of box B_1 is in series with the resistance of the thermostat and is extinguished each time this resistance is shorted. The flashing of this bulb indicates that the thermostat is functioning correctly with an accuracy of about $\frac{1}{50}^\circ$ C. The thermostat has been in continuous use for more than a year with no trouble. A resistance box R is used to obtain suitable sensitivity and critical damping of the galvanometer.

The thermostat is partly filled with water on top of which is poured a little oil to prevent evaporation. The constant temperature junction of the thermocouple is kept submerged in the water. The copper wire from this junction is connected to the resistance box *R* from which the circuit goes through the galvanometer and to switch S_w. The copper wire leading from this switch to the rectal applicator should be taken from the same spool as the other copper wire. The constantan wire leads from the thermostat to the binding post *P*. There it is directly connected to another constantan wire which again must be taken from the same spool in order that an additional thermo-electric effect may be avoided. The size of both the copper and constantan wires used here is *B* and *S* No. 30 (0.25 mm. diameter).

A rectal thermocouple applicator that is not subjected to the high temperature and humidity of a Kettering hypertherm machine can be simply constructed by soldering a thermocouple junction to the inside of a hollow copper tip that is screwed to a bakelite tube of shape similar to that of Fig. 2. The thermocouple leads go through this tube, and inside a single outer tubing, to the cold junction and measuring instruments. Enamel insulation of the wires is sufficient, in these cases, to prevent the wires from short circuiting.

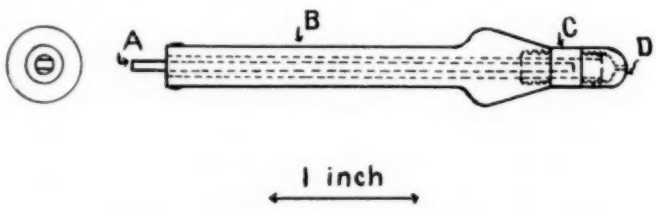

Fig. 2.—Rectal thermocouple applicator.

When the applicator is used in conjunction with a hypertherm machine, many difficulties are presented. Moisture and heat cause the insulation between the two wires to break down, forming additional parallel, high resistance junctions at points that are usually at high temperatures. Good thermal contact must be obtained between the thermocouple junction and the patient. (Fecal masses may thermally insulate the couple if the patient's temperature is changing rapidly.) The thermocouple wires must not be subjected to the mechanical stress imposed by restless patients. The applicator should be constructed so that the inside of the tube is hermetically sealed from the outside.

An applicator that has given good results is shown in Fig. 2. It consists of a copper collar, *C*, that is screwed into a bakelite tube, *B*. A copper tip, *D*, is screwed to the other side of the copper collar. Inside of the bakelite tube is pressed a small bakelite rod that had been filed flat on opposite sides. This rod should fit tightly into the tube and may be sealed to it by the application of a thin coat of shellac. It should be long enough to extend well into the copper collar, so that the thermocouple wires, which are kept separated by the rod, will have no opportunity for contact until they are at points of approximately the same temperature as the junction. In order to solder the couple to the tip, the enamel is removed from the wires just before they protrude from the small hole in the copper tip, a wet cloth is wrapped around the collar, and the wires are

soldered by a short application of a hot soldering iron. The small hole is sealed by the solder and any excess of material may be smoothed off with a file.

One wire leading from the applicator is threaded* through a rubber tubing about ⅛ inch outside diameter, the other wire is threaded through a good quality rubber tubing, about ¼ inch inside diameter. "Gas" tubing is suitable for the latter. The small tubing is then threaded through the larger tubing and pushed against *B*, the rod, *A*, penetrating into it. Shellac and many turns of strong thread wound over the junction of the rod and small tubing adequately seal the two wires from each other and hold the tube in place, the larger tubing is slipped over the applicator body for about ½ inch, the end of the rubber

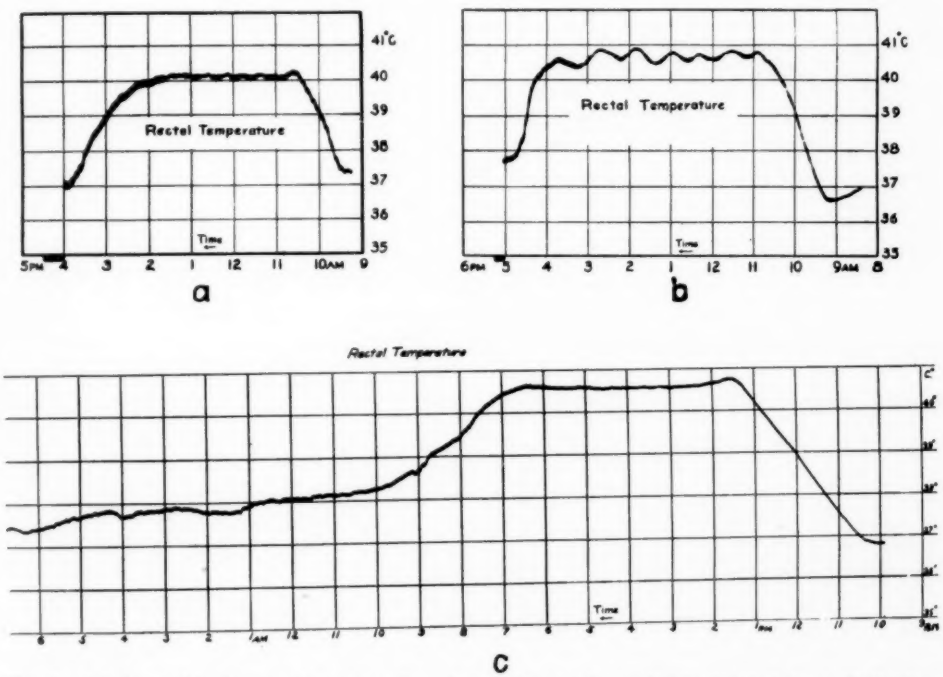

Fig. 3.—Photographic temperature records. *A* and *B* are for patients treated in a Kettering machine, *C* is for a patient treated by diathermy.

tubing is beveled so that thread may be wound to form a smooth junction between the applicator and tubing. The junction is shellacked.

Critical damping as well as suitable sensitivity of the galvanometer can be obtained by selecting proper parallel and series resistances. It is evident that the scale has to be calibrated in degrees. The thermostat is first set for a suitable temperature, e.g., 35° C. (95° F.). When the switch is open the light image should be directed to a proper line of the scale which then corresponds to 35° C. The rectal applicator is placed in water contained in a thermos bottle together with a calibrated thermometer and a few readings are taken at various temperatures within the range to be used. As the deflections are directly proportional to the temperature change over the range considered, only a few points on the

*A material may be quite easily threaded through a rubber tubing by first drawing through a piece of stiff wire (music wire is good) which may be used to pull through other materials.

scale need to be determined in this manner. In order to obtain reference points on the photographic chart it is only necessary to open the switch for short intervals at certain times. The dots thus obtained supply a time scale and the straight line drawn through them marks the 35° baseline. Fig. 3 *A* and *B* shows such complete records of the rectal temperature throughout the time the patients were kept in the Kettering hypertherm machine. These records are read from right to left. Fig. 3, *C* is a twenty-four-hour temperature record of a patient who was treated by diathermy while wrapped in blankets and rubber sheets. It is interesting to note that the temperature stayed well above normal throughout the night after the patient had been unwrapped. The sensitivity of the galvanometer had been regulated so that 1° C. corresponded to a deflection of about 2 cm.

The apparatus just described has proved to be of great value in connection with the hypertherm treatments. Any change in the rectal temperature of the patient is immediately reflected in the galvanometer reading and a glance at the scale gives the operator the needed information. The apparatus may get out of order, e.g., by the breaking of a wire. If that happens, it is fortunately easily discernible, and the ordinary rectal thermometer can be substituted until repair has been made.

We have described the apparatus at this time because of the need for such a device in connection with fever therapy. It was originally constructed for some special research problems. The apparatus can, of course, be used for studies of temperature fluctuations in different portions of the human body during twenty-four hours, and the photographic recording is of special value for such investigations.

NOTE.—A stranded copper wire with good rubber insulation can be substituted for the single copper wire and small rubber tubing. In this case the flattened bakelite rod should be omitted from the rectal applicator and the insulation of the copper wire should extend into the metal collar. We wish to thank Dr. Cook, of this hospital, for this suggestion.

A STAIN FOR URINARY CASTS*

JEANETTE ALLEN BEHRE, PH.D., CHICAGO, ILL., AND
WILLIAM MUHLBERG, M.D., CINCINNATI, OHIO

THE importance of urinary casts as prognostic evidence of cardiovascular renal disease has probably been neglected by the medical profession and the whole subject of these bodies appears to merit further study. Addis[1] has called attention to this point in his important contributions to the subject of this group of diseases and by his classification of Bright's disease he has fully demonstrated the value of a detailed study of urinary sediments.

Life insurance statistics indicate that casts, either hyaline or granular, and either intermittent or constant, herald the onset of cardiovascular renal disease about ten years before the cardiovascular renal apparatus is seriously affected. In other words, when casts are found in the urine the death rate appears normal during the first ten years, but after the tenth year the death rate is about 63 per cent above normal and the excessive deaths are largely accounted for by organic diseases of the heart, cerebral apoplexy, and Bright's disease. Evidently the age at which casts are first found is not of much significance. These statistics are based on a study of periods covering a total of 52,436 years, and their reliability appears more probable because two independent companies, with the material about equally divided between them, obtained almost parallel results.

A technic for staining urinary casts is described in this paper in the belief that it may prove useful in further study of the structure and composition of these bodies.

Various stains for casts have been suggested from time to time, especially iodine, picric acid, methylene blue, carmine, and eosin. We have found no reference in the literature to the use of methyl blue for this purpose. This dye, however, especially in combination with picric acid, brings out the detailed structure of casts and cast-like bodies in a remarkable way. It does not furnish a differential stain since all of the mucous material is also stained blue. It serves, however, to bring the casts and cast-like bodies into prominence and to reveal unsuspected details in their structure. The stain also makes photography of these bodies possible. The addition of eosin produces a contrasting color in many of the cellular structures of the urinary sediment.

With the technic described below hyaline casts are stained a clear blue of varying intensity. The more irregularly shaped bodies, sometimes classed as cylindroids, are similarly stained. An irregular distribution of material, a "mealy" structure, or a striated appearance sometimes becomes evident in bodies which appeared perfectly homogeneous before staining. Mucous threads and amorphous mucous material are also stained blue. They are clearly differentiated

*From the Laboratory of the Union Central Life Insurance Company, Cincinnati, and Northwestern University Medical School, Chicago.
Received for publication, August 7, 1936.

from the cast-like bodies by their structure. Granular material is usually stained darker. Mixed, finely granular casts present a striking picture of fine, dark granules powdered over the light blue, hyaline body. Some coarsely granular casts are stained deep blue, the granules of others are yellow, orange, or dark reddish brown. Renal epithelial cells are usually red, sometimes orange or yellowish. Red blood cells are stained a brighter red. Pus cells are usually red, occasionally blue. Epithelial cells from the urinary passages are either red or blue. Fat globules are unstained. In cells which are undergoing fatty degeneration, the fat globules are seen strikingly against reddish cellular material. The picric acid gives a light yellowish background.

Picric acid is used because when it is present the details of cast structure are clearer and the casts have a smoother, more natural appearance than in its absence. It has, however, the following disadvantages. (a) It complicates the microscopic picture by precipitating mucous material which is then stained. In our experience this almost never interferes with the study of casts, which stand out much more clearly than in unstained material.* In fact, in an occasional sample containing bacteria and débris and apparently no casts when unstained, the addition of the stain has revealed the presence of numerous casts. (b) Picric acid acts antagonistically toward eosin so that some, though not all, of the eosin-staining elements appear orange or yellow rather than red in its presence. However, if not too much picric acid is added the red color predominates in these bodies. The advantages of picric acid outweigh its disadvantages.

The use of this stain has suggested possible differences in structures otherwise undifferentiated. For instance, variations in the intensity with which "hyaline" or mixed casts are stained suggest differences either in consistency or in chemical composition. We have found casts containing renal epithelial cells imbedded in a "hyaline" matrix which take the stain more slowly and less intensely than the typical hyaline casts. On the other hand, some "hyaline" casts take the stain more deeply than usual. We are inclined to agree with Emerson[2] that a large group of casts exists which are more distinct and solid looking than hyaline casts but would not be classed as "waxy." It seems probable that in such casts there is a greater condensation of material than in the typical hyaline casts and that all gradations in such condensation can be found. The different staining reactions of coarsely granular casts and of pus and epithelial cells, mentioned above, may have to do with different stages in the disintegration of cellular material.

Often the stain reveals the presence of a lightly stained, mucus-like envelope, apparently covering the cast material. This envelope is often clearly seen at the end of a cast which has apparently been squarely broken off, or between two square-ended segments which appear to be in the process of separating from each other. The "tails" of the so-called cylindroids often appear to be the ends of these envelopes which may or may not contain thinned-out cast material. In-

*We have occasionally found a sample in which the casts had a shaggy appearance due possibly to the precipitation by the stain of large amounts of amorphous material. Reference to the unstained material and staining with a solution of methyl blue in water instead of picric acid are desirable in such cases. They do not occur frequently.

side the envelope the cast may appear with typically rounded ends, it may be convoluted or folded back on itself, or it may present segments of material more or less disconnected from each other.

The usual classifications of these bodies are probably too simple. On the other hand, some of the distinctions frequently made in the interests of morphologic classification appear to have little justification. For instance, an examination of these stained urinary sediments makes it difficult to believe that there is any reason for a sharp differentiation between the typical "cast," with rounded ends and parallel sides, and the other bodies, often called "cylindroids,"

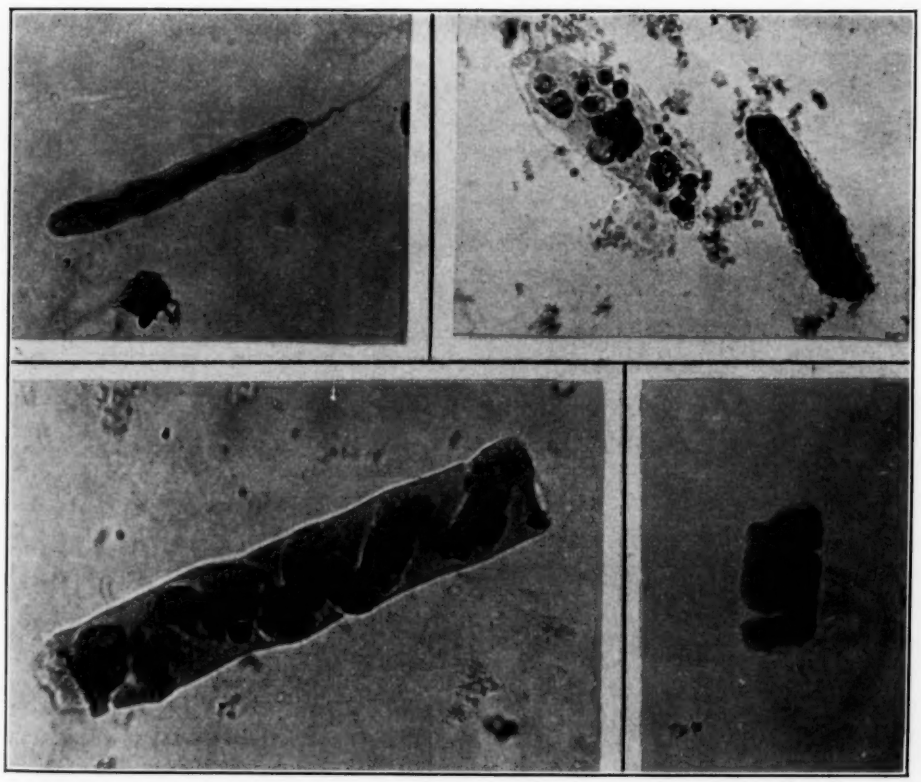

Fig. 1.

which do not conform to this shape. Flat, striated mucous threads or ribbons are unmistakably distinct from either of these types but all gradations in shape and in structure can be found between the typical, homogeneous "hyaline cast" on the one hand and the long-tailed, loosely packed, sometimes striated "cylindroid" on the other. The probable similarity of origin and significance of these bodies has been recognized[2-4] but in routine procedure an undue importance has often been attached to the "true cast."

Fig. 1 shows photomicrographs of several different types of casts stained with this stain.

STAINING TECHNIC

Solutions.—

1. A 0.5 per cent solution of eosin in water.
2. 1 c.c. of a 1 per cent aqueous solution of methyl blue added to 10 c.c. of saturated picric acid. The picric acid does not have to be particularly purified. We have used Merck's Picric Acid Reagent. About 10 drops of glycerol should be added for each 10 c.c. of picric acid.*

Method.—The urine is centrifuged and the supernatant liquid decanted as usual for microscopic examination. One drop of the eosin solution is added to the sediment and mixed by side to side shaking. After a minute or two 2 drops of the methyl blue picric acid solution are added and mixed. The color of the sediment should be distinctly blue green. If it is reddish brown, more of Solution 2 should be added until the blue green color is obtained, but too much is to be avoided. Some of the stained sediment is then transferred to a slide, covered with a cover glass, and examined microscopically. The amounts of the two stains added may be varied according to the amount of sediment present and to the particular character of the sediment. More eosin may be added if the cells have not been stained sufficiently red. Enough of the methyl blue should be present to stain the casts a distinct blue, but too much will stain them too dark.

More permanent slides may be made by adding more glycerol to the sediment and sealing the edges of the cover glass with vaseline or balsam.

We are indebted to Mr. George O'Connor, of the Union Central Life Insurance Company Laboratory, for his assistance in the study of this stain, and to Mr. Frederick Riehle, of the same Laboratory, for the photographs, taken with the photomicrographic apparatus which he constructed, with some modifications, according to the directions of Kennedy.[5]

We also wish to thank Professor Chester J. Farmer, of Northwestern University Medical School, for his suggestions in regard to this work.

REFERENCES

1. Addis, T.: A Clinical Classification of Bright's Diseases, J. A. M. A. **85**: 163, 1925.
2. Emerson, C. F.: Cylindruria, J. A. M. A. **46**: 5 and 89, 1906.
3. Endman, H. B.: The Origin of Tube-Casts, J. A. M. A. **59**: 1952, 1912.
4. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis, ed. 8, 1935.
5. Kennedy, J.: Making Your Own Photomicrographic Camera, Scientific American **152**: 76, 1935.

*Instead of methyl blue solution, 1.5 c.c. of blue black fountain pen ink (Waterman's, Carters, Skrip, etc.) added to 10 c.c. of saturated picric acid may be used. The dye used in these inks is evidently similar to methyl blue. The results with ink solutions are almost as good as those with methyl blue.

THE DETERMINATION OF CALCIUM IN CAPILLARY BLOOD*

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THE determination of the calcium content of the blood in infants is frequently desirable, but such determinations are rarely carried out in clinical work. Quantities of blood large enough for calcium determinations by the usual methods can only be obtained from the fontanel or external jugular vein. Methods which require only a few drops of blood from a lancet prick have been developed[1-7] but have been little used. In this paper results obtained with one of these methods, that of Nordbö,[1] are compared with those obtained with the Clark and Collip[10] modification of the method of Kramer and Tisdall;[13] and, using the Nordbö method, a comparison is made between the calcium values in blood obtained at the same time by lancet prick and by venous puncture.

The method of Nordbö, chosen because of its simplicity, is a modification of that of Trevan and Bainbridge[6] which in turn is similar to the earlier methods of Hirth[4] and of Lebermann.[5]

Methods.—The procedure used by us is as follows: Six or seven drops of blood from a skin puncture are collected in a centrifuge tube containing 0.1 unit of heparin. Care must be used to avoid an excess of the anticoagulant.[8] After centrifuging, the plasma is drawn off and 0.1 c.c. measured in a pipette calibrated "to contain." The pipette is washed with 0.1 c.c. of water, and the washings added to the sample. Two or three drops of saturated ammonium oxalate solution are now added, and the tube is allowed to stand for at least two hours. The precipitate is thrown down in the centrifuge and the supernatant fluid is carefully drawn off. Approximately 1 c.c. of 0.1 per cent ammonium oxalate is added as a washing fluid and the centrifuging is repeated. The supernatant fluid is once more removed and the tube is dried in an oven. When dry, it is placed in a sand-bath and the temperature is gradually brought to 200° or 250° C. By this treatment, the calcium oxalate loses its water of crystallization, and the excess ammonium oxalate is decomposed. After one-half hour the temperature is brought to 450° C. The tube is heated at this temperature for ten minutes, and then removed, and cooled in air. At this high temperature, the oxalate is completely converted to carbonate.[11]

For the titration of the carbonate, 0.1 c.c. of N/100 HCl is added to the calcium carbonate residue, and this is brought into solution by stirring with a fine glass rod. The tube is now heated for five minutes in a boiling water-bath, and then titrated with N/100 NaOH until the solution reaches a pH of 4.6 as shown by the indicator, bromeresol green. The endpoint is determined by com-

*From the Department of Biochemistry, University of Colorado School of Medicine.
Received for publication, August 8, 1936.

parison, against a white porcelain background, with a buffered standard at pH 4.6 containing the same amount of bromeresol green as is used in the unknown.

For throwing down the precipitates, use is made of ordinary 15 c.c. centrifuge tubes, which have been drawn out to a finer tip by heating in a Bunsen flame and allowing the tip to stretch out vertically when soft. The sand-bath for the incineration is about 8 cm. deep. The tube containing the dry calcium oxalate is thrust into it vertically so that its tip touches the bottom. A gas-filled mercury thermometer* capable of recording 550° C. is also thrust to the bottom beside the tube. Accurate titration is accomplished with a modified Rehberg buret,[9] having a capacity of 0.2 c.c.

In working with our subjects, all of whom were adults, we found it necessary to scrub the finger tip with a brush, using soap and water, before puncturing it. Otherwise, high calcium values were obtained apparently as a result of contamination of the blood with material from the skin.

Calculation of the calcium present in serum may be made by the following formula:

$$\text{mg. of Ca in 100 c.c. of sample} = (\text{c.c. of exactly 0.01 N HCl} - \text{c.c. exactly 0.01 N NaOH}) \times 200$$

Experimental.—The Nordbö technic was first checked on solutions of calcium chloride. Using 0.1 c.c. quantities of a carefully prepared calcium chloride solution containing 9.81 mg. of calcium per 100 c.c., the following yields in mg. of calcium per 100 c.c. were obtained:

9.70	9.50	9.90	9.31
10.09	9.70	10.09	9.70
9.70	9.70	9.11	10.09

Average = 9.72

The errors are somewhat larger than those reported by Nordbö (not more than 3 per cent), but certainly not large enough to invalidate the method as a clinical procedure.

Calcium was determined by the method of Nordbö on both venous serum and finger-prick plasma, obtained at the same time, and these were checked on the venous serum sample by the technic of Clark and Collip.[10] Table I summarizes the results of calcium determinations in a series of bloods from twenty normal subjects. The greatest variation between the venous serum calcium values on any one sample by these two methods was 0.84 mg. per 100 c.c. of serum with an average variation of 0.251 mg. The difference in the average calcium content of the twenty samples as determined by the two methods was 0.039 mg. per 100 c.c. of serum. A similar comparison of venous serum calcium (Nordbö method) and that of capillary plasma shows a maximum variation of 0.80 mg. per 100 c.c. of the sample with an average variation of 0.204 mg. The difference between the average of the twenty samples of capillary plasma and the twenty samples of venous serum was 0.116 mg. per 100 c.c. of the sample.

*Scientific Materials Company of Pittsburgh, Pennsylvania.

Discussion.—We feel that our results clearly establish the value of Nordbö's method for the determination of blood calcium as a clinical procedure. Not only is agreement found between results obtained on the same blood with Nordbö's method and the method of Clark and Collip, but with Nordbö's method close agreement is found between the calcium in blood obtained by finger prick and by venipuncture. This agreement between the calcium values found in venous and capillary blood is similar to that found by Smirk[12] who reports corresponding

TABLE I

COMPARISON OF THE CALCIUM CONTENT OF VENOUS SERUM (METHODS OF CLARK AND COLLIP AND OF NORDBÖ) WITH THAT OF CAPILLARY PLASMA (METHOD OF NORDBÖ)

BLOOD FROM PATIENT	(A) VENOUS SERUM METHOD OF CLARK AND COLLIP	(B) VENOUS SERUM METHOD OF NORDBÖ	(C) CAPILLARY PLASMA METHOD OF NORDBÖ	VARIATION BETWEEN (A) AND (B)	VARIATION BETWEEN (B) AND (C)
	mg. Ca per 100 c.c.	mg. Ca per 100 c.c.	mg. Ca per 100 c.c.	mg. Ca per 100 c.c.	mg. Ca per 100 c.c.
1	11.06	10.49	11.19	0.57	0.70
2	10.97	11.29	11.99	0.32	0.70
3	11.02	10.99	10.99	0.03	0.0
4	10.75	10.49	10.59	0.26	0.10
5	10.78	10.79	10.59	0.01	0.20
6	11.11	10.89	10.89	0.22	0.0
7	11.53	10.69	11.49	0.84	0.80
8	10.43	10.69	10.39	0.26	0.30
9	10.22	10.79	10.99	0.57	0.20
10	10.97	10.99	11.19	0.02	0.20
11	11.08	11.19	11.19	0.11	0.0
12	9.86	9.79	9.99	0.07	0.20
13	10.07	10.39	10.39	0.32	0.0
14	10.38	10.19	10.19	0.19	0.0
15	11.37	11.09	10.99	0.28	0.10
16	11.25	11.39	11.19	0.14	0.20
17	11.00	11.19	10.99	0.19	0.20
18	11.02	10.99	10.91	0.03	0.08
19	10.81	11.19	11.19	0.38	0.0
20	10.70	10.49	10.59	0.21	0.10
Average	10.819	10.78	10.896	0.251	0.204

Difference in averages of (A) and (B) = 0.039 mg. Ca per 100 c.c.
Difference in averages of (B) and (C) = 0.116 mg. Ca per 100 c.c.

values for iron and hemoglobin in blood obtained at the same time from these two sources. The conversion of the calcium oxalate to carbonate is apparently well controlled. Willard and Boldyreff[11] found quantitative conversion of 600 mg. of calcium oxalate to carbonate at 450° to 500° C. in one to two hours. It is not surprising, therefore, to find similarly complete conversion of such small quantities as 0.03 to 0.04 mg. at 450° C. in ten minutes. The single washing of the precipitated calcium oxalate with 0.1 per cent ammonium oxalate evidently does not introduce a significant error.

SUMMARY

1. Blood calcium was determined by the method of Nordbö on 0.1 c.c. of serum and by the method of Clark and Collip on 1 c.c. of the same serum. The calcium values thus obtained agreed within the limits of experimental error.

2. Determinations by the Nordbö method showed that the calcium concentration in capillary blood obtained by finger prick is the same as that in venous

blood. Hence, values obtained by the determination of calcium in blood from these two sources are directly comparable.

REFERENCES

1. Nordbö, R.: Mikro-Calciumbestimmung im Blute, Biochem. Ztschr. **246**: 460, 1932.
2. Groak, B.: Mikro-Calciumbestimmung im Serum und Plasma, Biochem. Ztschr. **212**: 47, 1929.
3. Fairhall, L. T., and Howard, R. G.: A General Method of Quantitative Microchemical Analysis, J. Roy. Microscop. Soc. **53**: 129, 1933.
4. Hirth, A.: Le Dosage du Calcium dans le Plasma Sanguin, Compt. rend. Soc. de biol. **88**: 458, 1923.
5. Lebermann, F.: Ueber eine neue klinische Methode der Mikrokalziumbestimmung im Blutserum, München. med. Wchnschr. **71**: 1392, 1924.
6. Trevan, J. W., and Bainbridge, H. W.: The Estimation of Calcium in Blood-Serum, Biochem. J. **20**: 423, 1926.
7. Rappaport, F., and Rappaport, D.: Volumetric Method for Determining Calcium in 0.2 Cubic Centimeter of Serum, Mikrochemie **15**: 107, 1934.
8. Van Bergen, T. M., and Hill, R. M.: The Effect of Added Heparin on Calcium Determinations in Blood Plasma, J. Lab. & Clin. Med. **22**: 862, 1937.
9. Longwell, B. B., and Hill, R. M.: A Modified Rehberg Burette for Use With Titrating Solutions Which React With Mercury, J. Biol. Chem. **112**: 319, 1935.
10. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, J. Biol. Chem. **63**: 461, 1925.
11. Willard, H. H., and Boldyreff, A. W.: The Determination of Calcium by Ignition of Calcium Oxalate to Carbonate in Air, J. Am. Chem. Soc. **52**: 1888, 1930.
12. Smirk, F. H.: The Effect of Water Drinking on the Blood Composition of Human Subjects in Relation to Diuresis, J. Physiol. **78**: 127, 1933.
13. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, J. Biol. Chem. **47**: 475, 1921.

A HOMEMADE ELECTRIC COUNTER FOR DIFFERENTIALS*

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With the modernizing of many hospitals, the house telephone is being replaced by the standard telephone system.

The electrical units of the house system can be utilized to advantage in making a blood counter for differentials. Such a counting apparatus (Fig. 1) was made in our hospital machine shop from a disused autophone system at practically no expense.

In the house system the connections between stations are made and maintained by a series of selectors each of which contains five magnets. By utilizing two of these magnets, the indicator for the individual count is controlled.

The indicator arm, which is the contact brush of the phone system, is attached to a ratchet which, in turn, is controlled by a selector magnet through its armature and pawl. The contact made in the key box causes the magnet to become energized and attract its armature, which causes the pawl to engage the ratchet and step it around one tooth for each attraction.

*From the Pathology Laboratory of the Newton Hospital.
Received for publication, August 11, 1936.

A second magnet is used as a release. When energized, this magnet attracts its armature, which releases the detent which holds the ratchet wheel. With this detent released, the ratchet returns to the original position through its mechanical spring. The release magnets are wired in parallel so that contact with the reset key causes a synchronous return of all indicators to their original position. These magnets, armatures, pawls, detent, and ratchet remain in the same position and relationship as in the original telephone selector. The wiring

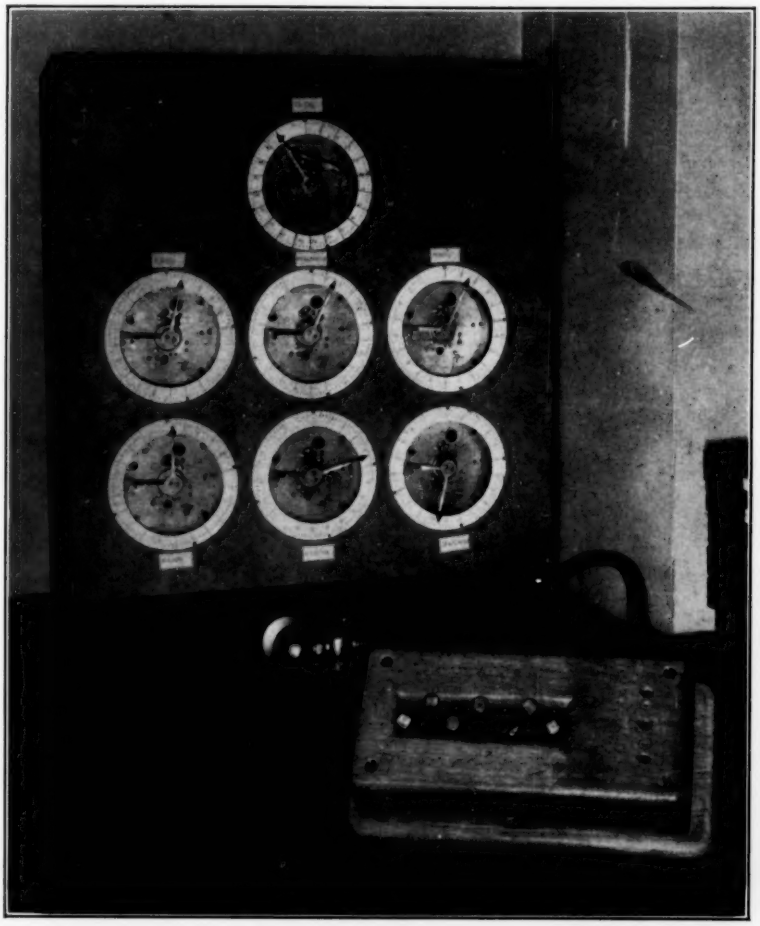

Fig. 1.

alone is changed. An arrow is soldered to the contact brush which completes the indicator arm. A third small magnet, one of the original five, is wired in parallel with the selector magnet to an independent circuit which is connected with the master indicator thereby giving the total count. The master indicator is similar in construction to the individual indicators with the exception that when the count has reached one hundred a contact arm closes a bell circuit.

The seven indicators are mounted in a box faced with wall board, the master indicator at the top. The diameter of the individual face is five and one-

half inches and can be read at a distance of five feet. The wires to the keyboard are contained in a six-foot movable cable, allowing perfect mobility of the keyboard. The number of individual selectors required will depend upon the white cell classification used. Dry cells are employed to operate the selectors. The apparatus shown in the figure has been in use for four months and has proved most satisfactory, reducing the time in making counts by nearly one-half.

THE EFFECT OF ADDED HEPARIN ON CALCIUM DETERMINATIONS IN BLOOD PLASMA*

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WHILE investigating methods for the determination of calcium in very small quantities of blood, it became evident that it would be preferable to use plasma rather than serum. There are two advantages in using plasma: (1) a greater volume of plasma can be obtained; (2) there is a shorter interval between the drawing of the blood and the time when the analysis may be carried out. If plasma is to be used, an anticoagulant must be chosen which does not influence the analysis. Heparin would seem to be an excellent anticoagulant for this purpose if it fulfills this condition. A review of the literature fails to answer the question. Greisheimer and Arnold,[1] and Holt[2] report higher values for calcium in heparinized plasma than in serum; Cantarow,[3] in a series of 100 determinations, reports lower values for calcium in heparinized plasma than in serum; Loucks and Scott[4] found that heparinized plasma gave results that did not differ from those obtained on serum more than the experimental error of the method employed. All of these investigators used the Clark and Collip[5] modification of the Kramer and Tisdall[6] technic. Because of this lack of agreement, we felt that this question should be reinvestigated.

Experimental.—In our study seven different samples of heparin were used. Four were prepared in this laboratory; two were from different lots prepared by Hynson, Westcott and Dunning, Inc., Baltimore, Maryland; and one, of high potency, was prepared in the Connaught Laboratories, Toronto, Canada.† We have no information on the method by which the commercial samples (No. 5 and 6, Table I) were prepared. Of our samples, No. 1, 2, and 4 were prepared according to the method of Howell[7] from dog liver, and No. 3 by the method of Charles and Scott[8] from beef liver. Sample 7, from the Connaught Laboratories, was, presumably, prepared by the method of Charles and Scott,[8] and purified by the method of Scott and Charles.[9] The activity of the different samples was estimated by the method of Charles and Scott.[8]

Equal quantities of blood from one individual were added to a series of tubes containing dry, powdered heparin in amounts varying from 0.01 to 20.0

*From the Department of Biochemistry, University of Colorado School of Medicine.
Received for publication, August 8, 1936.
†This sample was kindly furnished by Professor D. A. Scott.

mg. per 5 c.c. of blood. The calcium contents of these different plasmas were determined by the acidimetric micromethod of Nordbö[10] and by the permanganate titration method of Clark and Collip.[5] Since the values obtained were in good agreement the averages only are reported. The results are summarized in Table I.

Heparin 1, when present in quantity about ten times the amount necessary to prevent coagulation, reduces the calcium value about 40 per cent; Heparin 4 in the same weight concentration reduces the calcium value 12 to 15 per cent; while Heparin 2 shows a tendency to raise the calcium value slightly. Heparin

TABLE I

THE INFLUENCE OF HEPARIN UPON THE DETERMINATION OF BLOOD CALCIUM

	HEPARIN IN MG. PER 5 C.C. OF BLOOD		0.0 (SERUM)	0.01	0.1	1.0	2.0	4.0	10.0	16.0	20.0
Calcium content of plasma in mg. per 100 c.c.	Heparin 1	Blood 1	10.35			10.15	10.06	7.70	6.46		
	0.7 unit per mg.	Blood 2	10.08			9.77			5.80		
	Heparin 2*	Blood 1	12.00			11.99	12.30	12.20	12.45		
	0.0 unit per mg.	Blood 2	11.26			11.76	12.09	12.30	12.39		
	Heparin 3	Blood 1	10.85			11.35	11.18	11.34	10.50		
	0.5 unit per mg.	Blood 2	11.33					10.10	10.79	10.0	9.60
	Heparin 4	Blood 1	11.55			11.09	11.09	11.19	9.80		
	0.6 unit per mg.	Blood 2	10.35					11.39	9.09	8.34	7.89
	Heparin 5	Blood 1	10.39			11.59	11.89	12.39	13.19		15.28
	1.2 unit per mg.										
	Heparin 6	Blood 1	11.79			12.00	12.00	12.00	12.40		12.10
	1.2 unit per mg.										
	Heparin 7	Blood 1	10.08	9.98	9.98	10.59			11.20		
	100 units per mg.										

*This sample had very little activity. A small clot appeared in all but the last tube in Blood 1. In Blood 2, 1 mg. per 5 c.c. of Heparin 1 was added to each tube.

samples 5 and 6 (Hynson, Westcott and Dunning), though more active than the samples made in our laboratory, cause increases in the calcium value when used in excess. When 20 times the minimal quantity of Heparin 5 is used (20 mg.), an increase of 40 per cent in the calcium value is produced. The increase caused by 1,000 times the minimal quantity of Heparin 7 (10 mg.) is only 12 per cent. With the exception of Heparin 2 all of the samples made in this laboratory, when used in excess, reduce the calcium value. All four samples are student preparations and were made at about the same time. The three made by the method of Howell (1, 2, and 4) are identical in appearance; but though Heparin 1 is fairly active, Heparin 4 is less active and Heparin 2 has practically no activity.

Discussion.—In evaluating these data it must be remembered that, with the exception of Heparin 7, the active substance is a very small part of the bulk of the preparation. It is quite possible that the effect on the calcium determination is produced by material which is inert so far as blood coagulation is concerned. This is almost certainly true in the case of a rise in the calcium value, as occurs with Heparin samples 5 and 6, for there is no relation between this rise in calcium and the amount of active heparin present. When an increase in calcium follows the addition of heparin to a blood sample, we feel that calcium must have been present as an impurity in the reagents used in its preparation. When a fall in calcium occurs, this effect seems to be proportional, at least roughly, to the

activity of the heparin sample. The samples prepared in this laboratory arranged in descending order of their activity as anticoagulants (1, 4, 3, and 2) are found also to be in descending order of their activity in decreasing the calcium precipitated from blood by oxalate. The preparation of a heparin which causes a fall in the calcium value was entirely fortuitous on our part, and no record of the reagents employed was kept. It seems possible, however, that our reagents were unusually free from calcium and that this is the reason the heparin prepared with them shows the properties reported here. The results obtained when Heparin 7 from the Connaught Laboratories was used closely approximate a balance between these two extremes. This sample produces neither a rise nor a fall in calcium when moderate amounts are used, and even with 100 times the minimal amount only a slight increase in the calcium value occurs. A consideration of the foregoing brings us to suggest that, in preparing heparin, if little or no calcium is present in the reagents, correspondingly little will be present in the product. Any excess of such a heparin used beyond that necessary to prevent clotting will remove calcium from the blood sample until it is "saturated." If just enough calcium is present in the reagents to "saturate" the heparin, then no matter how much excess of such a "saturated" heparin is used the calcium value will remain unchanged.

It is evident from the data in Table I that in most cases even two or three times the usual minimal quantity of heparin might be used without appreciable influence on the calcium value, but that in some cases (Heparin 5) there may be enough calcium contamination to make the sample unsuitable for use in such determinations. Any new sample of heparin to be used for this purpose should be tested by determining the effect of an excess on the determination of blood calcium.

All the samples of heparin tested with the exception of the relatively pure sample from the Connaught Laboratories, Heparin 7, contained such large amounts of inorganic phosphate as to render them unsuitable for use when phosphate is to be determined.

SUMMARY

1. The effect of excess heparin has been studied in the determination of plasma calcium.

2. Some samples of heparin used in excess produce a rise in the calcium value; others produce a fall.

3. When a rise is produced, it is apparently due to contamination of the heparin sample.

4. When a fall is produced, it appears to be related to the activity of the heparin preparation.

5. It is suggested that the difference in samples of heparin is due to differences in the calcium content of the reagents used in their preparation.

6. When heparin is to be used in preparing plasma for determination of calcium, the effect of adding an excess of the preparation should be tested.

7. Heparin, except when highly purified, cannot be used when phosphate is to be determined in the blood.

REFERENCES

1. Greisheimer, E. M., and Arnold, A. W.: Blood Chemistry Changes in Children Produced by Exposure to the Alpine Lamp, Am. Rev. Tubere. **14**: 479, 1926.
2. Holt, G. W.: Heparin in Blood Calcium Analyses, Proc. Soc. Exper. Biol. & Med. **29**: 315, 1932.
3. Cantarow, A.: Calcium Metabolism and Calcium Therapy, Philadelphia, 1931, Lea and Febiger, p. 36.
4. Loucks, M. M., and Scott, F. H.: Calcium in the Coagulation of the Blood, Am. J. Physiol. **91**: 27, 1929.
5. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, J. Biol. Chem. **63**: 461, 1925.
6. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, J. Biol. Chem. **47**: 475, 1921.
7. Howell, W. H.: Heparin, an Anticoagulant, Am. J. Physiol. **63**: 434, 1923.
8. Charles, A. F., and Scott, D. A.: Studies on Heparin. I. The Preparation of Heparin, J. Biol. Chem. **102**: 425, 1933.
9. Scott, D. A., and Charles, A. F.: Studies on Heparin. III. The Purification of Heparin, J. Biol. Chem. **102**: 437, 1933.
10. Nordbö, R.: Mikro-Calciumbestimmung im Blute, Biochem. Ztschr. **246**: 460, 1932.

A DIABETIC KIT*

H. E. FAVER, M.D., BUFFALO, N. Y.

FOR some time it was felt that the necessity for a compact and portable diabetic kit existed. To be useful, the kit should be small, self-contained, and the outfit should include an insulin syringe, a needle, Benedict's solution, and facility for sterilizing syringe and needle and for testing the urine. Various types of electric heaters were tried, and as most laboratory manuals stated that the Benedict's solution should be boiled vigorously during the test, the tube containing this solution was heated directly by the electric element. In every case, this direct heating of the tube caused violent bumping and expulsion of the solution. Various methods were tried to control this, such as wire gauze surrounding the tube, bits of wire gauze within the tube, glass beads, and small pieces of agate, without success. Another factor that militated against the usefulness of an apparatus which heated the test fluid directly was the fact that the syringe and needle could not be satisfactorily sterilized in this manner and that other provisions had to be made to keep the needle and syringe continually immersed in alcohol.

It was then decided to test the efficiency of Benedict's solution when the tubes were immersed in boiling or simmering water and kept at a temperature of approximately 200 degrees F. for various periods. To have a control in testing this method, it was decided to construct a small cylindric boiler, electrically heated, which would contain from one to four small test tubes, and use it in rontine urine tests. It was decided to test this boiler in the laboratory of the Buffalo State Hospital. The routine urines would be subjected to two tests, one by

*From The Buffalo State Hospital.
Received for publication, September 23, 1936.

boiling the Benedict's solution with a gas flame in the orthodox manner, and the other test by immersing the test tube containing the Benedict's solution and urine in a simmering boiler. In the boiler the same quantity of reagents and urine was used and kept immersed in the boiler where the water was kept constantly simmering for a period of five minutes. The simmering water in all cases was at the level of, or slightly above, the Benedict's solution in the test tubes.

The results of the tests that were run in duplicate are shown in the appended table and consist of 88 urines; they were the routine urines that were examined

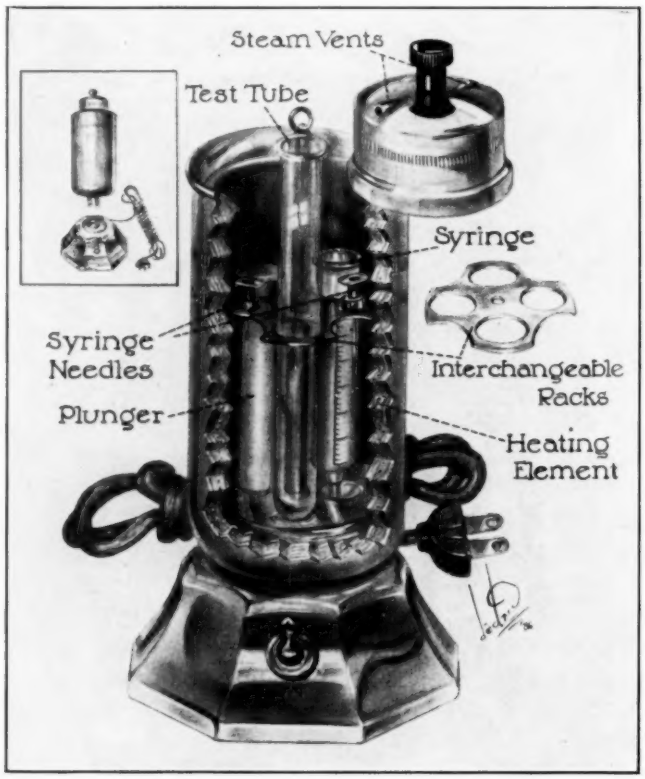

Fig. 1.

in the laboratory of the Buffalo State Hospital from April 30 to May 11, 1936, inclusive. The results in all cases were exactly identical, indicating that the reactions would go to completion if the tubes containing the Benedict's reagents and urine were immersed in simmering water at 200 degrees F., or more, for five minutes.

Of 88 urines tested routinely, 3 showed positive sugar in both the diabetic kit and in the usual manner of laboratory testing; 3 showed trace of sugar in both types of testing; and 82 were negative in both types of testing.

As the kit is constructed,* it contains a small rack with four openings, so that 1 to 4 tubes may be tested at the same time, or 1 tube, syringe, and needle

*This kit is constructed by the Gomco Surgical Manufacturing Corporation, 87 Ellicott Street, Buffalo, N. Y.

boiled at the same time. In the construction of the boiler, it was decided to decrease the length of time within which water was brought to a boil, as in the ones that heated more rapidly, there was some spluttering of water. It was found that if the water was kept slowly simmering results were quite satisfactory.

The cooperation of Elmer L. Sevringhaus, of the University of Wisconsin, in the early development of this instrument is gratefully acknowledged. Also the cooperation of L. M. Green, pathologist at the Buffalo State Hospital, is gratefully acknowledged for the performance of the laboratory tests.

THE EXTRACTION OF CHOLESTEROL FROM BLOOD*

H. J. Rose, and Cecilia Riegel, Philadelphia, Pa.

THE desirability of a uniform method of analysis for free and combined cholesterol in both bile and blood led to a study of the applicability of the digitonide method described by us[1] to three methods of extraction of whole blood, serum, or plasma. The methods of extracting cholesterol from blood were (1) Bloor[2] alcohol ether extraction; (2) Schoenheimer-Sperry[3] alcohol acetone extraction; (3) direct extraction of the whole blood, serum, or plasma with ether and magnesium sulphate, as described for bile in a previous paper.[1]

In both (1) and (2) the extraction is carried out as described by the authors. Subsequently the digitonin precipitation is made on a suitable aliquot.

In (3) 1 c.c. of oxalated whole blood or plasma (or serum) is diluted to 10 c.c. with water. One cubic centimeter of 15 per cent magnesium sulphate is added, and the mixture then shaken vigorously for twenty minutes with three successive 100 c.c. portions of ether. The ether fractions are decanted into a 500 c.c. Erlenmeyer flask, the ether distilled off to a small residue (1 to 3 c.c.) and the residue taken up in alcohol-acetone (1:1) as in the bile analysis.

Table I

SAMPLE	DILUTION	EXTRACTIONS	CHOLESTEROL	
			TOTAL	FREE
	c.c.		mg. %	mg. %
Human whole blood	3 to 100	Alcohol Ether	184	104
Human whole blood	5 to 100	Alcohol Ether	190	104
Human whole blood	5 to 50	Alcohol Acetone	185	103
Human serum	2 to 25	Alcohol Acetone	215	---
Human serum	1 each	Multiple Ether	217	---
Dog whole blood	5 to 100	Alcohol Ether	144	104
Dog whole blood	5 to 50	Alcohol Acetone	152	104
Dog whole blood	1 each	Multiple Ether	149	99
Dog plasma	5 to 50	Alcohol Ether	123	48
Dog plasma	5 to 50	Alcohol Acetone	121	48
Dog plasma	1 each	Multiple Ether	121	44

*From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania.

Received for publication, August 28, 1936.

For free cholesterol, digitonin precipitation is made directly on the alcohol acetone extract. For total cholesterol the extract is saponified by adding 0.1 c.c. of 33 per cent potassium hydroxide and heating two and one-half hours at 38° C. The extract is then acidified and precipitated with digitonin.

Subsequent analysis is as described for bile.[1]

Mean values are reported in Table I.

SUMMARY

Cholesterol, free and combined, is extracted from whole blood, plasma, or serum equally well by single extractions made with alcohol ether or alcohol acetone, or by multiple extracts with ether.

REFERENCES

1. Riegel, C., and Rose, H. J.: Determination of Free and Combined Cholesterol in Bile, J. Biol. Chem. **113**: 117, 1936.
2. Bloor, W. R.: The Determination of Cholesterol in Blood, J. Biol. Chem. **24**: 227, 1916.
3. Schoenheimer, R., and Sperry, W.: A Micro Method for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**: 745, 1934.

A METHOD FOR DETERMINING THE HEART RATE OF SMALL ANIMALS*

MICHAEL KNIAZUK, RAHWAY, N. J.

THE counting of the heart rate of small animals with a frequency of more than 200 beats per minute cannot be accomplished accurately by auscultation or palpation, but requires special methods of recording. Usually an electrocardiogram is taken and the number of beats per minute calculated from the photographic record. This makes it impossible to obtain the results instantly; although the method is accurate, it is impractical for routine determinations on a large scale.

In the method to be described, the photokymograph, together with the electromagnetic oscillograph, is replaced by a cathode ray oscillograph. Visual observations of the electrocardiogram on the fluorescent screen of the cathode ray tube are made by connecting the amplified heart action current to the vertical deflecting plates and a sweep circuit oscillator to the horizontal plates. If the frequency of the sweep is the same as that of the heart rate, a stationary picture of the electrocardiogram will result, whereas a difference in frequency of the two currents will cause a drifting of the wave to the right or left. In this way readings of the heart rate can be made at the instant of synchronization, provided the frequency of the sweep circuit is known. The ordinary type of sweep circuit using a gaseous discharge tube is not sufficiently constant in its frequency output to be calibrated and used for this purpose. The relaxation oscillator, or multivibrator, is a much more constant frequency generator,

*From the Merck Institute of Therapeutic Research.
Received for publication, August 11, 1936.

and is used in this case to trip the sweep circuit. The circuit of the multivibrator is shown in Fig. 1. With the given values it vibrates approximately 200 to 800 times per minute. When all four condensers C_1, C_2, C_3, C_4 are in the circuit, the lower half of the range between 200 and 400 vibrations is obtained. By throwing the switches S_1 and S_2, condensers C_2 and C_3 are disconnected and the upper range from 400 to 800 is covered. The variable resistances R_1 and R_2 which are on the same shaft serve to adjust the frequency within the two ranges mentioned above. The dial on these resistances is calibrated to read in number of vibrations per minute by using the 60 cycle A.C. as a stand-

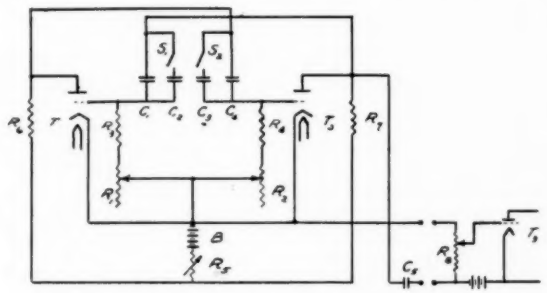

Fig. 1.

$B = 135$ volts
$C_1, C_2, C_3, C_4 = 0.1$ mfd.
$C_5 = 0.004$ mfd.
$R_1, R_2, R_3, R_4 = 200{,}000$ ohms
$R_5 = 10{,}000$ ohms
$R_6, R_7 = 20{,}000$ ohms
$R_8 = 100{,}000$ ohms
$T_1, T_2 =$ type 76 tube
$T_3 =$ discharge tube of sweep circuit.

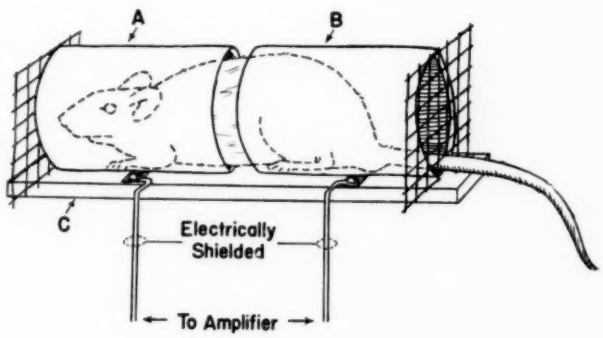

Fig. 2.

ard. The apparatus is operated by batteries; this simplifies the setup and eliminates any possibility for the oscillator to lock with the A.C. due to the low frequency at which it operates.

Resistance R_5 is used to compensate for any change in battery voltage and is adjusted once before each set of readings in the following manner: A.C. is connected to the vertical plates of the cathode ray tube and the dial set to a point where a multiple of the sine wave should be stationary. If the wave tends to drift it is brought to a standstill by means of R_5. It is essential that the frequency of the thyratron circuit be lower than that of the relaxation oscillator, otherwise the sweep will return before it is tripped by the multivibrator.

For taking the electrocardiogram, the animal is placed in a special holder (Fig. 2). The animal rests on two wet cloth pads *A* and *B* with his fore and hind legs. The pads are re-enforced internally with sheet metal and fastened to the bakelite strip *C*. The leads are not typical for electrocardiography but serve well the purpose of obtaining an action current of the heart. The cage must fit the animal snugly, and therefore different sizes are required for guinea pigs, rats, mice, etc.

The principal advantages of the method are:

1. Accurate measurements of the heart rate (±5 beats per minute) can be obtained within one or two minutes.

2. No anesthetic or sedative is necessary to quiet the animal, since a distortion of the wave, caused by sudden struggling, is easily recognized as such.

3. By using instead of needle electrodes the special holder described, excitement of the animal is to a large extent overcome. This is especially important for securing consistent and accurate results in bradycardia tests.

The above method has been in daily use in our laboratory for more than three months for the testing of vitamin B_1 avitaminosis on rats, and has been found very satisfactory.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEAD POISONING, A Modified Stain for Stipple Cells in, Mc Kinney, R. A., and Rosenzweig, S. J. A. M. A. **106:** 1660, 1936.

The three reagents required are kept for convenience in covered Coplin jars. The technic is as follows:

Fix dried smear in acetone-free methyl alcohol for from three to five minutes and transfer directly to Wright's stain (staining time predetermined for each lot of stain used); wash in tap water and transfer to dilute ammonia water (2.5 c.c. of stronger ammonia water in 1,000 c.c. of distilled water); dip up and down rapidly until blue color runs from slide; wash in tap water, dry and examine.

The finely stippled or coarsely dispersed "basophilic aggregations" in the red blood cells appear distinctly black against the gray or pink of the stained cell. The white blood cells retain the usual nuclear stain.

The ratio of lead-affected cells to the normal red cells is determined by an adaptation of the Fonio platelet counting technic. A minute opening in a paper disk dropped into the ocular diaphragm gives a suitable counting field. The stipple cells and the normal red cells in each field are counted, but tabulated in separated columns, until 250 normal red cells have been counted. The number of stipple cells is then multiplied by 4, giving the ratio of such cells to 1,000 normal cells. The number of thousands of red blood cells per cubic millimeter multiplied by the number of stipple cells per thousand cells gives the approximate number of lead stipple cells per cubic millimeter of blood.

This staining method offers nothing fundamentally new; its chief advantage lies in the fact that a simplification and combination of two previous technics makes possible:

1. A rapid detection and enumeration of stipple cells in a spread which is also adequately stained for a differential count of the white blood cells.
2. The utilization of reagents usually found in any physician's office or clinical laboratory.
3. A technic which stains dried smears two months old as satisfactorily as fresh dried smears.

LYMPHOGRANULOMA INGUINALE, Cultivation of the Virus in Mice and Its Use in the Preparation of Frei Antigen, Grace, A. W., and Suskind, F. H. Arch. Dermat. & Syph. **33:** 853, 1936.

A strain of the virus of lymphogranuloma inguinale obtained from the pus and glandular material of a patient presenting the inguinal type of the disease has been successively transmitted in mice by intracerebral inoculation for 41 passages (at the time of writing).

The virus increased in virulence with successive passages in mice.

Lymphogranulomatous mouse brains provided a readily available source of specific Frei antigen, the potency of which increased with successive passages.

Frei antigens prepared from mouse brains were tested six months after preparation and found to retain their potency for at least that length of time.

Emulsions of normal mouse brains prepared and tested in the same way as Frei antigen did not produce any appreciable reaction.

In the 143 Frei tests done on 22 lymphogranulomatous human subjects and the 145 tests done on 38 normal human subjects, no untoward effects were caused by the use of material prepared from mouse brains.

The brains of mice which died from an intracerebral inoculation of the virus of lymphogranuloma inguinale showed meningoencephalitis on histologic examination. Exudate into the meninges and ventricular system and perivascular infiltration with infrequent formation of abscesses in the brain substance were the chief lesions.

The inflammatory cells consisted of polymorphonuclear leucocytes, macrophages, plasmacytoid cells, a few small lymphocytes and a number of very large cells, possibly macrophages. The proportion in which the cells of the first three types occurred depended on the length of time that elapsed between inoculation and death.

Intracytoplasmic bodies were encountered infrequently.

LYMPHOGRANULOMA, Frei Test With Antigens Made From Mouse Brain, Strauss, M. J., and Howard, M. E. J. A. M. A. **106**: 517, 1936.

Some change occurs in antigens made from mouse brain within a few weeks after preparation which, when injected intradermally, gives rise to a reaction almost indistinguishable from a true positive reaction.

The nature of this change is at present unknown.

This occurs in antigens made from the brains of normal mice as well as in antigens made from the brains of mice inoculated with lymphogranuloma inguinale.

The false reaction is induced in normal subjects as well as in patients with lymphogranuloma inguinale.

For this reason Frei antigens made from mouse brain would not appear to be suitable for the routine diagnosis of lymphogranuloma inguinale.

BLOOD: Values for Red Blood Cells of Average Infants and Children, Mugrage, E. R., and Andresen, M. I. Am. J. Dis. Child. **51**: 775, 1936.

The quantity of hemoglobin, the number of red blood cells, and the volume of packed cells have been determined accurately on samples of venous blood from children ranging in age from birth to thirteen years.

The children are divided into eighteen groups according to age, and the mean values are reported for each group. Differences in blood values between boys and girls are not considered to be especially significant. Averages for the amount of hemoglobin, the number of red blood cells, and the volume of packed cells which are high at birth reach a low level in the period between the ages of two and four months. There is a transient rise between the ages of four and eight months, followed by a second decrease. The sustained increase begins at the age of eighteen months and continues to the twelfth year. Values for the twelve-year-old children are practically identical with those for the normal women of our previously reported series.

The mean corpuscular hemoglobin content, volume and hemoglobin concentration are compared with mean values for normal adults of our series.

The means for corpuscular hemoglobin and volume are highest at birth and in the first few days of life. The lowest mean for corpuscular volume appears in the period between the ages of twelve and eighteen months, and the adult level is reached in the three-year-old group.

The corpuscular hemoglobin concentration remains at a remarkably uniform level throughout the period of life covered in the present study. The majority of values for children of all ages fall within the range observed for the blood of normal adults.

SPLENECTOMY, Blood Picture After, in Children, Wollstein, M., and Kreidel, K. V. Am. J. Dis. Child. **51**: 765, 1936.

In forty-four children the blood picture following splenectomy varied with the cause for the removal of the spleen.

When there had been no preoperative diminution in the number of platelets, as in cases of traumatic rupture, rheumatic disease, and congenital hemolytic icterus, there was no im-

mediate increase after the operation; but an increase appeared toward the middle or end of the first week, rarely before the third day.

In cases of hemorrhagic thrombocytopenia, in which the number of platelets had been much diminished, splenectomy was followed by an immediate rise in all but one instance. This rise was not maintained in a fatal case and was delayed in the case of a boy who has lived two and one-half years after operation. The peak was reached, as a rule, in the second postoperative week, and a level of 1,000,000 platelets per cubic millimeter was maintained for a week or for several months.

A fall in the reticulocyte count was characteristic of typical cases of congenital hemolytic icterus.

In patients with Cooley's anemia, the response of the platelets was most irregular in onset, number, and duration of the increase, nor was it ever as high or of as long duration as in the other groups. Increase in the number of nucleated red cells is characteristic in these cases.

Operations other than splenectomy were not followed by any appreciable rise in the platelet count during the postoperative period of observation.

TYPHOID FEVER, Rapid Slide Test for the Serological Diagnosis of, Welch, H., and Mickle, F. L. Am. J. Pub. Health 26: 248, 1936.

A rapid slide test for the diagnosis of typhoid and paratyphoid fevers is presented which requires approximately 4 minutes to complete. The results obtained in a study of 1,100 serums indicate that the slide test is at least as sensitive and specific as the tube tests with which it was compared. The stability of the antigens used, the speed and accuracy with which a serum may be analyzed by this method, and the consistency of the results obtained, indicate that the test should be an excellent one for use in public health laboratories and in hospitals where an early exclusion of typhoid fever in certain undiagnosed cases is often important.

The method is as follows:

On a 5 by 7 glass slide five rows of small wax rings are placed (made as in the conduct of Kline precipitation tests).

The patient's serum is pipetted in the following amounts: 0.08, 0.04, 0.02, 0.01, 0.005, and 0.002 ml. in the first four rows, using a Kahn pipette (0.2 ml. graduated in thousandths). In the fifth row (used only for the first test each day) 0.08 ml. of 0.85 per cent salt solution is added to each of the four rings for controls on each of the four antigens. A drop of "O" antigen is added to each of the serum amounts in the first row and to the first ring in the fifth row. Similarly, a drop of "H" antigen is added to each of the serum amounts in the second row and to the second ring in the fifth row. *S. paratyphi* is added to the third row and *S. schottmuelleri* to the fourth row, each with the appropriate controls in the third and fourth rings, respectively, in the fifth row. The above serum-antigen mixtures in each row correspond to dilutions of 1:20 through 1:640 in the tube test. All antigens are shaken gently but well before using. Each row of serum-antigen mixture is thoroughly mixed with a separate toothpick or applicator starting with the smallest amount (0.002 ml.) of serum and working from right to left. After mixing, the glass slide is gently rocked back and forth 15 or 20 times and the degree of clumping estimated. The type of agglutination obtained with the "O" antigen does not correspond to the typical small flaking or granular agglutination obtained in the tube test, and hence "O" and "H" agglutination cannot be differentiated by appearance. This is no disadvantage since both types of antigens are used.

PHOSPHATASE ACTIVITY, Serum Calcium, Inorganic Phosphorus and, Guttman, A. B., Tyson, T. L., and Gutman, E. B. Arch. Int. Med. 57: 379, 1936.

The calcium and inorganic phosphorus content of the serum and the serum phosphatase activity were determined in 4 cases of hyperparathyroidism, 76 cases of Paget's disease, 6 cases of multiple myeloma and 45 cases of neoplastic disease of the bones. The relevant data from the determinations in the differential diagnosis of the diseases of bone under consideration are discussed.

1. Of the cases of hyperparathyroidism in which determinations are recorded in the literature, about four-fifths showed consistent hypercalcemia, with a calcium content of more than 12 mg. per 100 c.c., and about one-half, consistent hypophosphatemia, with an inorganic phosphorus content of less than 2.5 mg. Increase in blood phosphatase activity was found in every case of classic hyperparathyroidism with definite changes in the bones in which determinations were made (28 cases).

2. The calcium and inorganic phosphorus contents of the serum are within normal limits in cases of uncomplicated Paget's disease. The serum phosphatase activity is almost invariably increased in cases of advanced disease reaching values forty or more times the mean normal value. The level for serum phosphatase is roughly proportional to the extent of involvement of the bones and is probably affected also by the activity of the osseous lesions. In this sense, the determination may be of value in prognosis. Increased blood phosphatase activity is not specific for Paget's disease. The increase in instances of early, localized Paget's disease is often equivocal, so that the determination is of little value in such cases. Nor is it usually of assistance in differentiating cases of osteoplastic metastases in the bone, in which serum phosphatase activity is also increased.

3. Definite hypercalcemia has been observed in a number of cases of multiple myeloma. The inorganic phosphorus content of the serum is normal or somewhat elevated. The serum phosphatase activity is usually normal or slightly elevated.

4. Hypercalcemia is present in occasional cases of carcinoma with extensive, predominantly osteolytic metastases to the bones. The amount of inorganic phosphorus in the serum is usually within normal limits, but it may be depressed in cases of advanced cachexia or increased when renal insufficiency is present. The serum phosphatase activity is usually moderately increased or essentially normal.

In cases of carcinoma with extensive, predominantly osteoplastic metastases, the serum phosphatase activity may be as high as that in advanced Paget's disease. The content of calcium and that of inorganic phosphorus in the serum are normal, except when renal insufficiency is present. In cases of carcinoma with metastases to the liver, with or without jaundice, variable increases in serum phosphatase activity are observed.

LEUKOCYTES, Rest and Activity Levels of, in Health and Disease, Medlar, E. M. Arch. Int. Med. **57**: 367, 1936.

Definite "rest" and "activity" levels of the leucocyte count do not exist if the activity is limited to mild exercise.

Complete relaxation, such as a night's sleep or two hours in bed, appears to bring about a more irregular distribution of the leucocytes, both in the total and in the differential count, than is present during a state of normal activity.

Great care should be exercised whenever an attempt is made to interpret the significance of the leucocytic picture when the fluctuations noted are within a normal range.

Fluctuations occur in abnormal as well as in normal leucocytic pictures.

These variations, when allowed for, do not seriously interfere with the pathologic interpretation of the significance of leucocyte counts.

BLOOD COAGULATION, Factors Involved in, in the Newborn Infant: IV. Variations in Fibrinogen Content, Crane, M. M., and Sanford, H. A. Am. J. Dis. Child. **51**: 99, 1936.

The quantity of fibrin formed in the plasma of infants during the first ten days of life has been studied. The average value for fibrin under the conditions of this investigation was found to be 0.38 gm. per 100 c.c. of plasma, with variations between 0.22 and 0.67 gm. In the great majority of cases the content was between 0.25 and 0.55 gm. There is commonly a rise in the amount of fibrin during the first three to five days of life, after which there is no consistent change.

From further studies (Ibid. **51**: 311, 1936) the same authors conclude that:

Assuming, as it seems safe to do, that the determination of fibrin gives a satisfactory index of the plasma fibrinogen, the experimental results obtained show that injections of the mother's whole blood do not increase the fibrinogen content of the plasma in the normal newborn infant. It seems improbable, therefore, that the beneficial effect of such injections in the case of hemorrhagic disease of the newborn infant is due to increased formation of fibrinogen.

BLOOD, Occult, in Stools of the Newborn, Bonar, B. E. Am. J. Dis. Child. **51**: 255, 1936.

Occult blood is found too frequently in the stools of newborn infants to be attributed to parental sources or to gross lesions of the alimentary tract.

Investigation of other possible etiologic factors reveals that it is not influenced by sex, frequency of stools, initial loss of weight (dehydration) or the hemoglobin and erythrocyte levels. It is not responsible for the characteristic reduction of the levels of these constituents of the blood during the first ten days of life.

Occult bleeding is influenced by the weight at birth, by the kind and amount of food to some extent by the duration of labor. An explanation is offered for the decreased amount of bleeding found in infants delivered by operation.

The observations of some authors that the incidence of occult blood in the stools is increased when jaundice is present could not be fully corroborated. The relationship noted in this study between bleeding and jaundice was found to be more apparent than real.

Prolongation of coagulating and bleeding times, as well as the tendency for the blood of the newborn to hemolyze, are definite contributing factors in occult bleeding. However, the bleeding seems to be more closely related to the bleeding time, and hence to the condition of the vessel walls, than to the clotting elements of the blood.

Occult bleeding is probably more dependent on the early bacterial invasion of the alimentary tract than on the type of organism involved.

The observations recorded lend support to the previously mentioned assumption that the presence of occult blood in the stools is the result of hyperemia of the mucosa. However, this explanation fails to take into consideration the fact that blood is detected before the irritation by bacteria or food can be responsible. The following mechanism, which begins with birth, seems plausible: In the first two or three days the vascular engorgement from pressure and asphyxia during the birth process results in the loss of blood either by rupture of minute mucosal vessels or through increased permeability of their walls. In the next few days bleeding is increased temporarily by the intense hyperemia set up by bacteria and food. This is probably augmented by a prolongation of the clotting and bleeding times at this time and possibly by an increased permeability of the vessel walls. With the subsidence of some of these factors there is a corresponding reduction in the amount of bleeding, but this may be delayed somewhat by the rapid rise in consumption of food.

UNDULANT FEVER, Due to B. Suis, Horning, B. G. J. A. M. A. **105**: 1978, 1935.

An outbreak of undulant fever with fourteen cases and three deaths occurred in a home for elderly persons.

Raw milk from the institution herd was the only source of infection found.

The herd consisted of thirty-six cows and one bull. The blood from two cows was positive and from another suggestive for brucella infection.

Brucella suis was isolated from the blood of two patients and from an abscess of a third patient.

Blood was drawn from thirty-two swine kept by the institution. Nine were positive and seven suggestive for brucella infection.

The cattle had opportunity for natural infection from the swine.

Epidemiologic evidence suggests that the outbreak was due to *Brucella suis* received from drinking raw milk from cows infected with *Brucella suis* from swine.

CORRESPONDENCE

The Disappearance of Phenols and Cresols Added to "Biological Products" on Standing

Dear Sir:

In the paper with the above title* the following statement was made:† "After a certain length of time, definite for each type of product (expiration date), the vaccine, antitoxin (or other product) is returned to the producer, retested for potency, and if found satisfactory and sterile, revialed and redistributed for use." This statement is true in part only. It is incorrect so far as vaccines are concerned. Vaccines, if returned, are not at any time redistributed by a producer. Antitoxins, if returned, may be reprocessed and then, if satisfactory, may be revialed and redistributed.

Attention may also be called to the interesting paper‡ by P. Masucci and M. I. Moffat in which the loss of phenol and tricresol in biological products when stored at room temperature in rubber-capped vials because of the diffusion of the phenol or tricresol vapors through the rubber caps was described.

K. George Falk.

Dear Sir:

In connection with our article on Pulmonary Moniliasis which appeared in the April issue, p. 687, we should like to give credit to our laboratory technician, Rachel Milos, for the routine laboratory work done in this connection.

Earl L. Warren.

*This JOURNAL 22: 641, 1937.
†Page 642.
‡J. Am. Pharm. Assn. 12: 117, 1923.